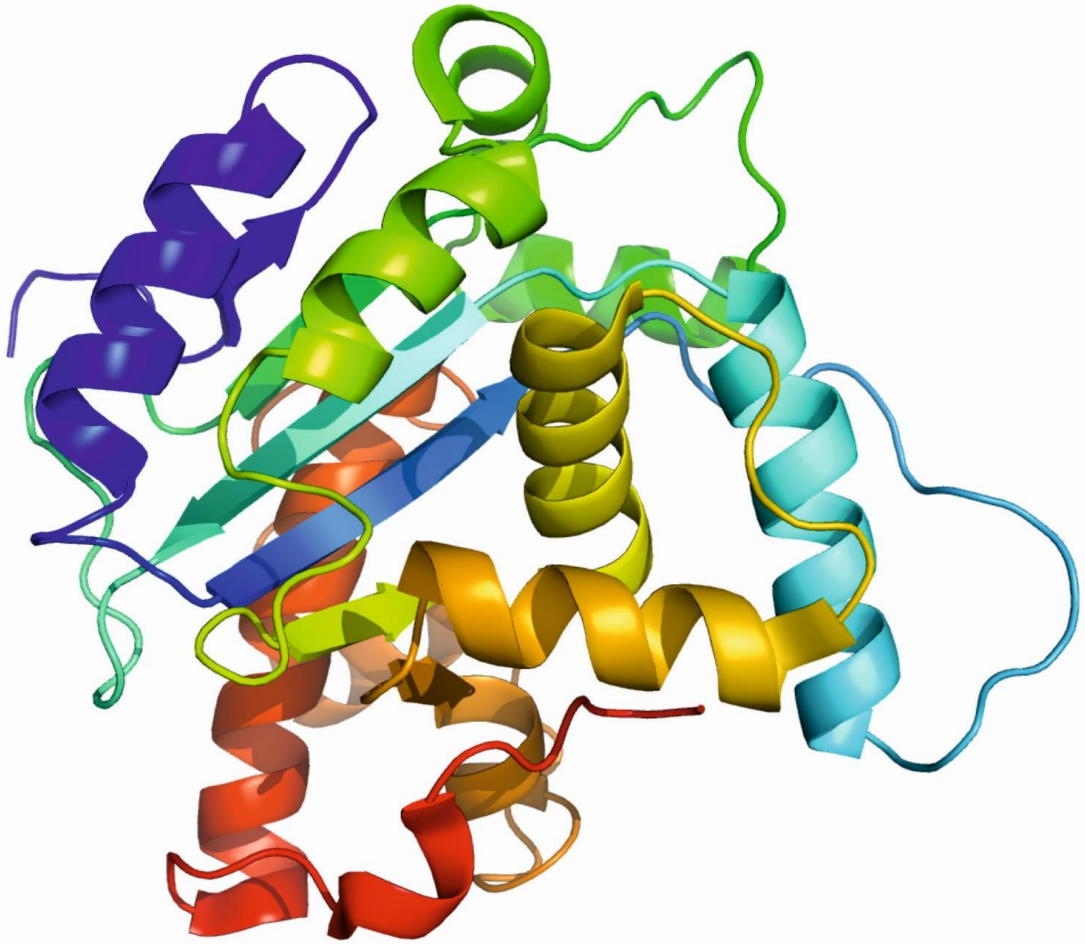
Biochemistry



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Biochemistry

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PREFACE

Since this is the first book of this series, we have witnessed the birth of simple solutions to comparatively bigger file of Biochemistry. Consequently, the basic sciences are becoming more important to the practice of Pharmacy. Medicine and science. This puts a new pressure on the student to understand the basis of molecular medicine and the molecular bio sciences in terms of biochemistry. We still think that it's easier to remember things that you understand, things that make sense. That's the idea behind the Basic Concepts series and that's why we have been so pleased with the expansion of the Basic Concepts series beyond Biochemistry.

The book topics are related to the explosion of new information about bio molecules, proteins, amino acids and related diseases of human beings along with their prognosis. In addition, we have added some tables of information that I think will be helpful in seeing the big picture (and remembering some of the more important details). The major topics and things to remember are set off in boxes so that if you already know everything in the box, you can skip the rest of the section.

Acknowledgments

I am grateful to my institute Sasnkriti University, Mathura India and our Hon Chancellor Sir Shri Sachin Gupta Ji for supporting me in the field of research and innovation. I am grateful to my research guide during the M. Pharm. course Prof. Sanjay B. Bari at R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule, Maharashtra and Dr Anil K Saxena former Deputy Director and Head MPC Division, CSIR-CDRI Lucknow, India who generously shared their expertise to help make this book as accurate and as useful to Pharmaceutical, Medical (and biomedical graduate) students as possible. I am particularly appreciative of the support and encouragement provided by my institute collogues for providing me an invaluable and healthy environment throughout the process. I am fortunate to have my PhD students Ms. Deepti Mathpal and Mr. Mukesh Masand whose continues efforts for this book made it possible to publish the book in simplest format for all students.

I am thankful for the efforts of the production house editors and production staff of K D Publication Pune, Maharashtra India. Many, many thanks to Dr. Rajni Adam for providing me opportunity to write a book and freedom during the writing of book.

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Dedication

The Book is dedicated to My Father, Guides, Friends and Students

This book is dedicated to the memory of my father late. Shree Mohansing, my god father of research and mentor of current institute Dr Anil K Saxena whose efforts in shaping my research and carrier make this book possible. I will not be able to write this book without the committed efforts of my wife Dr Neha and my notorious kids Vihaa and Viraaj for their love of drawing the figures in my system during the completion of book.

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Chapter 1

Bioenergetics

1.1 Introduction:

Bioenergetics means study of the transformation of energy in living organisms. The goal of bioenergetics is to describe how living organisms acquire and transform energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics. In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy. Adenosine triphosphate (ATP) is the main "energy currency" for organisms; the goal of metabolic and catabolic processes are to synthesize ATP from available starting materials (from the environment), and to break- down ATP (into adenosine diphosphate (ADP) and inorganic phosphate) by utilizing it in biological processes. In a cell, the ratio of ATP to ADP concentrations is known as the "energy charge" of the cell. A cell can use this energy charge to relay information about cellular needs; if there is more ATP than ADP available, the cell can use ATP to do work, but if there is more ADP than ATP available, the cell must synthesize ATP via oxidative phosphorylation. Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is hydrolyzed (broken down by water) to adenosine diphosphate and inorganic phosphate. Here it is the thermodynamically favorable free energy of hydrolysis that results in energy release; the phosphoanhydride bond between the terminal phosphate group and the rest of the ATP molecule does not itself contain this energy.

1.2 Types of Bioenergetics Reactions:

1.2.1 Exergonic Reaction:

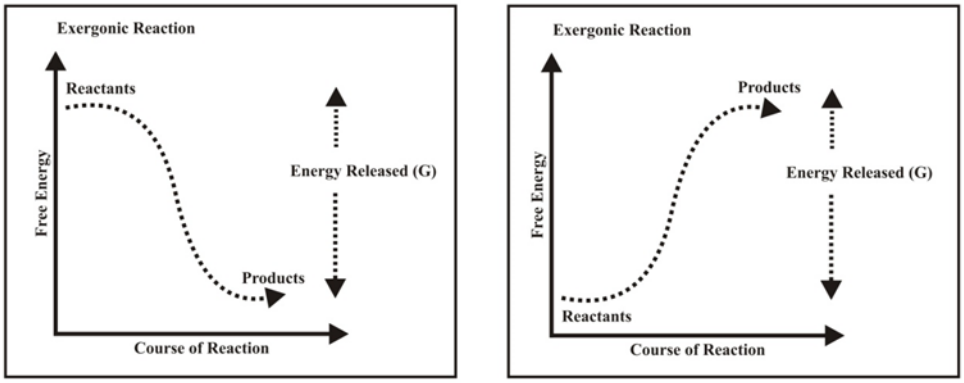
a. Exergonic implies the release of energy from a spontaneous chemical reaction without any concomitant utilization of energy.
b. The reactions are significant in terms of biology as these reactions have an ability to perform work and include most of the catabolic reactions in cellular respiration.
c. Most of these reactions involve the breaking of bonds during the formation of reaction intermediates as is evidently observed during respiratory pathways. The bonds that are created during the formation of metabolites are stronger than the cleaved bonds of the substrate.
d. The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as

$$\Delta G = G_{products} - G_{reactants} < 0$$

1.2.2 Endergonic Reactions:

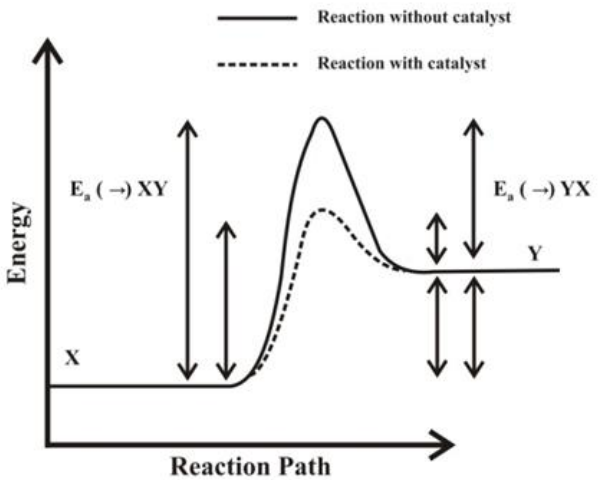
a. Endergonic in turn is the opposite of exergonic in being non-spontaneous and requires an input of free energy.
b. Most of the anabolic reactions like photosynthesis and DNA and protein synthesis are endergonic in nature.
c. The release of free energy, G, in an exergonic reaction (at constant pressure and temperature) is denoted as

$\Delta G = G_{products} - G_{reactants} > 0$



1.2.3 Activation Energy:

a. Activation energy is the energy which must be available to a chemical system with potential reactants to result in a chemical reaction. Activation energy may also be defined as the minimum energy required starting a chemical reaction.



I. Examples of Major Bioenergetics Processes:

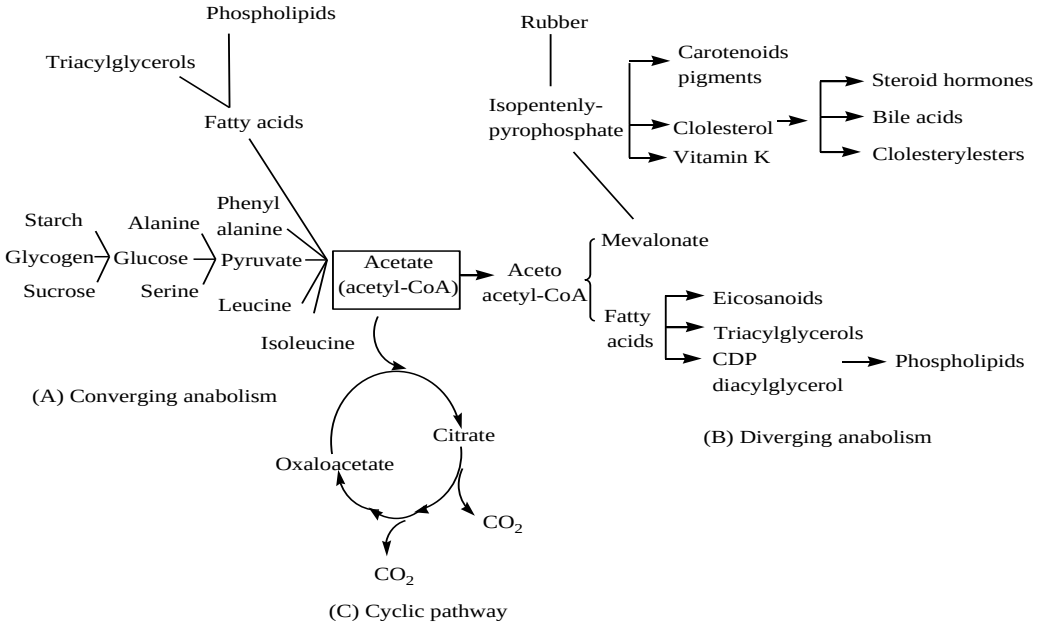
Glycolysis is the process of breaking down glucose into pyruvate, producing net eight molecules of ATP (per 1 molecule of glucose) in the process. Pyruvate is one product of glycolysis, and can be shuttled into other metabolic pathways (gluconeogenesis, etc.) as needed by the cell.

Additionally, glycolysis produces equivalents in the form of NADH (nicotinamide adenine dinucleotide), which will ultimately be used to donate electrons to the electron transport chain. Gluconeogenesis is the opposite of glycolysis; when the cell's energy charge is low (the concentration of ADP is higher than that of ATP), the cell must synthesize glucose from carbon- containing biomolecules such as proteins, amino acids, fats, pyruvate, etc. For example, proteins can be broken down into amino acids, and these simpler carbon skeletons are used to build/ synthesize glucose.

The citric acid cycle is a process of cellular respiration in which acetyl coenzyme A, synthesized from pyruvate dehydrogenase, and is first reacted with oxaloacetate to yield citrate.

The remaining eight reactions produce other carbon- containing metabolites. These metabolites are successively oxidized, and the free energy of oxidation is conserved in the form of the reduced coenzymes $FADH_2$ and NADH.

These reduced electron carriers can then be re- oxidized when they transfer electrons to the electron transport chain. Ketosis is a metabolic process whereby ketone bodies are used by the cell for energy (instead of using glucose). Cells often turn to ketosis as a source of energy when glucose levels are low; e.g. during starvation.



The electron transport chain and Oxidative phosphorylation are the processes where decreasing equivalents such as NADPH, $FADH_2$ and NADH were used to contribute electrons to a series of oxidative and reductive reactions that take place in electron transport chain complexes. These type of redox reactions take place in mitochondrial membrane inside the enzyme complexes. These reactions transfer electrons "down" the electron transport chain, which is coupled to the proton motive force.

The difference between proton concentration in the mitochondrial matrix and internal membrane space is used to uplift ATP synthesis via ATP synthase.

In plants the photosynthesis of energy using glucose, solar energy and cabondioxide is another major bioenergetic process, for metabolism. The reaction undegoes in the chloroplast. Once the glucose is synthesized, the plant cell can undergo photophosphorylation to produce ATP.

II. Bioenergetics Relationship between Free Energy, Enthalpy & Entropy

a. Every living cell and organism must perform work to stay alive, to grow and to reproduce. The ability to harvest energy from nutrients or photons of light and to channel it into biological work is the miracle of life.
b. 1^{st} Law of Thermodynamics: The energy of the universe remains constant.
c. 2^{nd} Law of Thermodynamics: All spontaneous processes increase the entropy of the universe.
d. The important state functions for the study of biological systems are:

i. The Gibbs free energy (G) which is equal to the total amount of energy capable of doing work during a process at constant temperature and pressure.

- If ΔG is negative, then the process is spontaneous and termed exergonic.
- If ΔG is positive, then the process is nonspontaneous and termed endergonic.
- If ΔG is equal to zero, then the process has reached equilibrium.

ii. The Enthalpy (H) which is the heat content of the system. Enthalpy is the amount of heat energy transferred (heat absorbed or emitted) in a chemical process under constant pressure.

- When ΔH is negative the process produces heat and is termed exothermic.
- When ΔH is positive the process absorbs heat and is termed endothermic.

iii. The Entropy (S) is a quantitative expression of the degree of randomness or disorder of the system. Entropy measures the amount of heat dispersed or transferred during a chemical process.

- When ΔS is positive then the disorder of the system has increased.
- When ΔS is negative then the disorder of the system has decreased.

IV. The conditions of biological systems are constant temperature and pressure. Under such conditions the relationships between the change in free energy, enthalpy and entropy can be described by the expression where T is the temperature of the system in Kelvin.
$\Delta G = \Delta H - T\Delta S$

[ΔG = *Gibbs free energy*; ΔH = *Change in Enthalpy*; T = *Temperature in* K; ΔS = *Change in Entropy*]

THREE THERMODYNAMIC QUANTITIES

QUANTITY	SYMBOL	MEASURES	UNITS
Enthalpy	H	Heat	Energy
Entropy	S	Disorder	Energy/K
Free Enetgy	G	Reactivity	Energy

1.2.4 Energy Rich Compounds:

a. High energy phosphates act as energy currency of cell.
b. Three major sources of high energy phosphates taking part in energy conservation or energy capture.

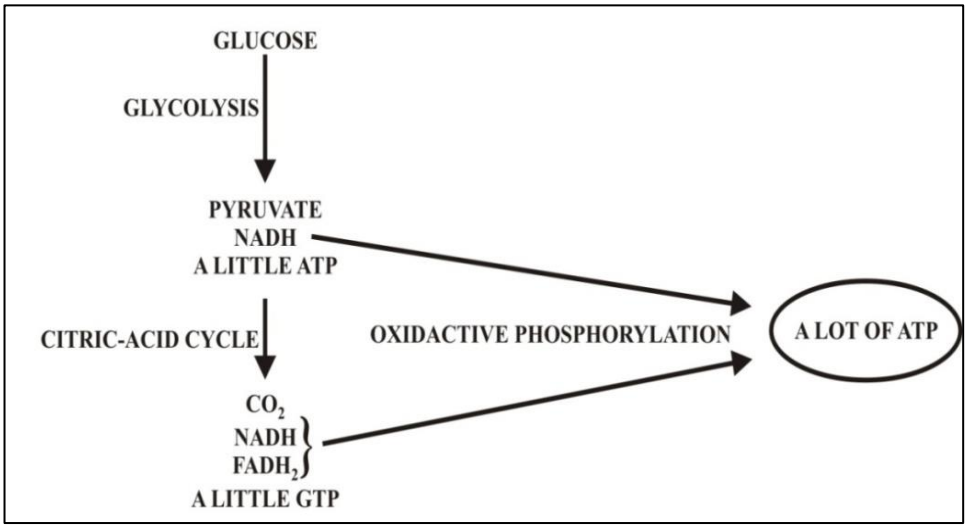
I. Oxidative Phosphorylation (or OXPHOS in short):

a. In metabolic pathway, cells use enzymes to oxidize nutrients, thereby releasing energy which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation.

   This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.

b. The process that accounts for the high ATP yield is known as oxidative phosphorylation.
c. In glycolysis and the citric-acid cycle generate other products besides ATP and GTP, namely NADH and $FADH_2$.

   These products are molecules that are oxidized (i.e., give up electrons) spontaneously. The body uses these reducing agents (NADH and $FADH_2$) in an oxidation-reduction reaction



1.2 Glycolysis:

Cells use the glycolysis pathway to extract energy from sugars, mainly glucose, and store it in molecules of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH). The end product of glycolysis is pyruvate, which can be used in other metabolic pathways to yield additional energy. During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

	Reactions Catalyzed	**ATP used**	**ATP formed**
Stage I	1. Glucokinase (for phosphorylation) 2. Phosphofructokinase I (for phosphorylation)	1 1	
Stage II	3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain) 4. Phosphoglycerate kinase (substrate level phosphorylation)		6 2
Stage III	5. Pyruvate kinase (substrate level phosphorylation)		2
	Total	2	10

In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

I. TCA Cycle:

a. The citric acid cycle (CAC) – also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl- CoA derived from carbohydrates, fats, and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP).

b. If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

S. NO.	REACTIONS	NO. OF ATP FORMED
1.	2 Isocitrate ⟶ 2 α-Ketoglutarate ($2\ NADH + 2H^+$) (2x3)	6
2.	2 α-Ketoglutarate ⟶ 2 Succinyl CoA ($2\ NADH + 2H^+$) (2x3)	6
3.	2 Succinyl CoA ⟶ 2 Succinate (2 GTP = 2 ATP)	2
4.	2 Succinate ⟶ 2 Fumarate ($2\ FADH_2$) (2x2)	4
5.	2 Malate ⟶ 2 Oxaloacetate ($2\ NADH + 2H^+$) (2x3)	6
	TOTAL NO OF ATP FORMED	24

- **Energy shuttles:**

i. **NADH:** An energy shuttle which delivers high energy electrons to the electron transport chain where they will eventually power the production of 2 to 3 ATP molecules. When this electron shuttle is not carrying high energy electrons, meaning it has been oxidized (lost its electrons), it is left with a positive charge and is called NAD^+.

ii. **$FADH_2$:** Another energy shuttle that carries high energy electrons to the electron transport chain, where they will ultimately drive production of 1 to 2 ATP molecules. The oxidized form of $FADH_2$ is FAD and happens just like in NADH.

High energy molecules:

iii. **ATP:** The basic energy currency of the cell. It's a form of energy that cells can use right away.

GTP: Similar to ATP, GTP can be easily converted to ATP in the cell.

1.3. Energy Released by Hydrolysis of Some Phosphate Compounds:

1.3.1 Adenosine Triphosphate (ATP):

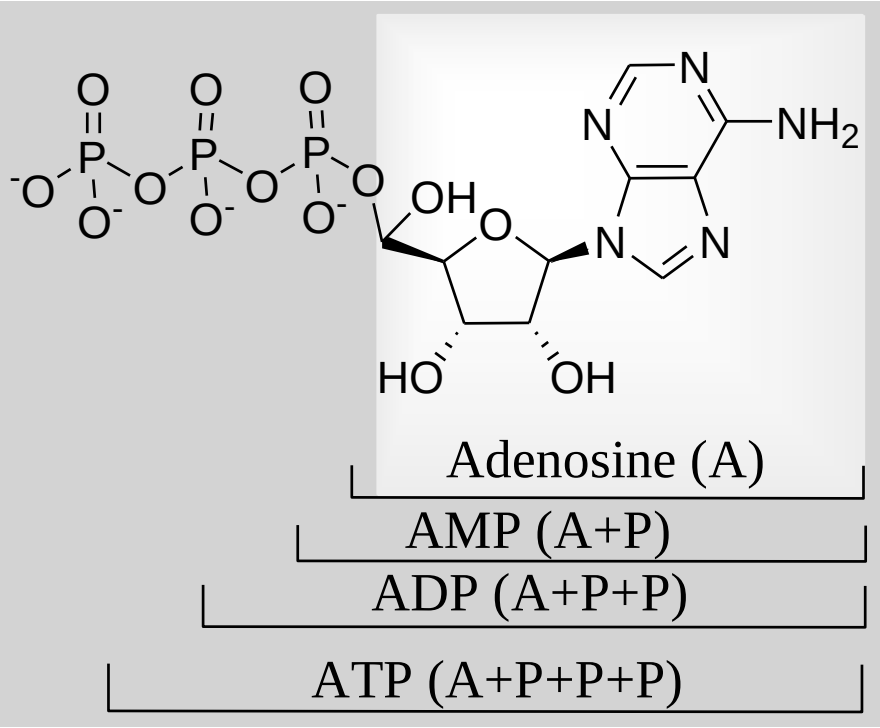
- The multifunctional nucleotide in cells called Adenosine-5'-triphosphate (ATP) is used as a coenzyme.

- ATP is called as functional unit of energy for intracellular energy transfer. It carries the chemical energy within cells for metabolism.
- ATP id manufactured by photophosphorylation and cellular respiration and utilized for many cellular processes, including biosynthetic reactions, motility, and cell division by enzymes and structural proteins.
- ATP synthase produces one molecule of ATP from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP) which contains three phosphate molecules.

The structure of this molecule consists of a purine base (adenine) attached to the 1' carbon atom of a pentose sugar (ribose).

Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP.

When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.



A. The three main functions of ATP in cellular function are:

i. Transporting organic substances—such as sodium, calcium, potassium—through the cell membrane.
ii. Synthesizing chemical compounds, such as protein and cholesterol.
iii. Supplying energy for mechanical work, such as muscle contraction.

- The amount of energy released during the hydrolysis of ATP can be calculated from energy changes under non-natural (standard) conditions, correlated to biological concentrations. The released energy by cleavage of phosphate (Pi) or pyrophosphate (PPi) unit from ATP at standard state of 1 M are:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^\circ = -30.5 \text{ kJ/mol } (-7.3 \text{ kcal/mol})$$

$$ATP + H_2O \rightarrow AMP + PP_i \quad \Delta G^\circ = -45.6 \text{ kJ/mol } (-10.9 \text{ kcal/mol})$$

These values can be used to analyze the change in energy under physiological conditions and the cellular ATP/ADP ratio (also known as the Energy Charge). The reaction is directly dependent on number of factors, including overall ionic strength and the presence of alkaline earth metal ions such as Mg^{2+} and Ca^{2+}. In controlled conditions ΔG is approximately −57 kJ/mol (−14 kcal/mol).

1.3.2 Cyclic Adenosine Monophosphate:

(cAMP, cyclic AMP or 3'-5'-cyclic adenosine monophosphate)

cAMP is derived from ATP and is a second messenger system important in many biological processes.

It is used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway.

It is produced from ATP by adenylyl cyclase located inside the plasma membrane. Adenylyl cyclase is activated by signaling molecules through the activation of stimulatory G (Gs)-protein-coupled receptors of adenylyl cyclase and inhibited by agonists of adenylyl cyclase inhibitory G (Gi)-protein-coupled receptors.

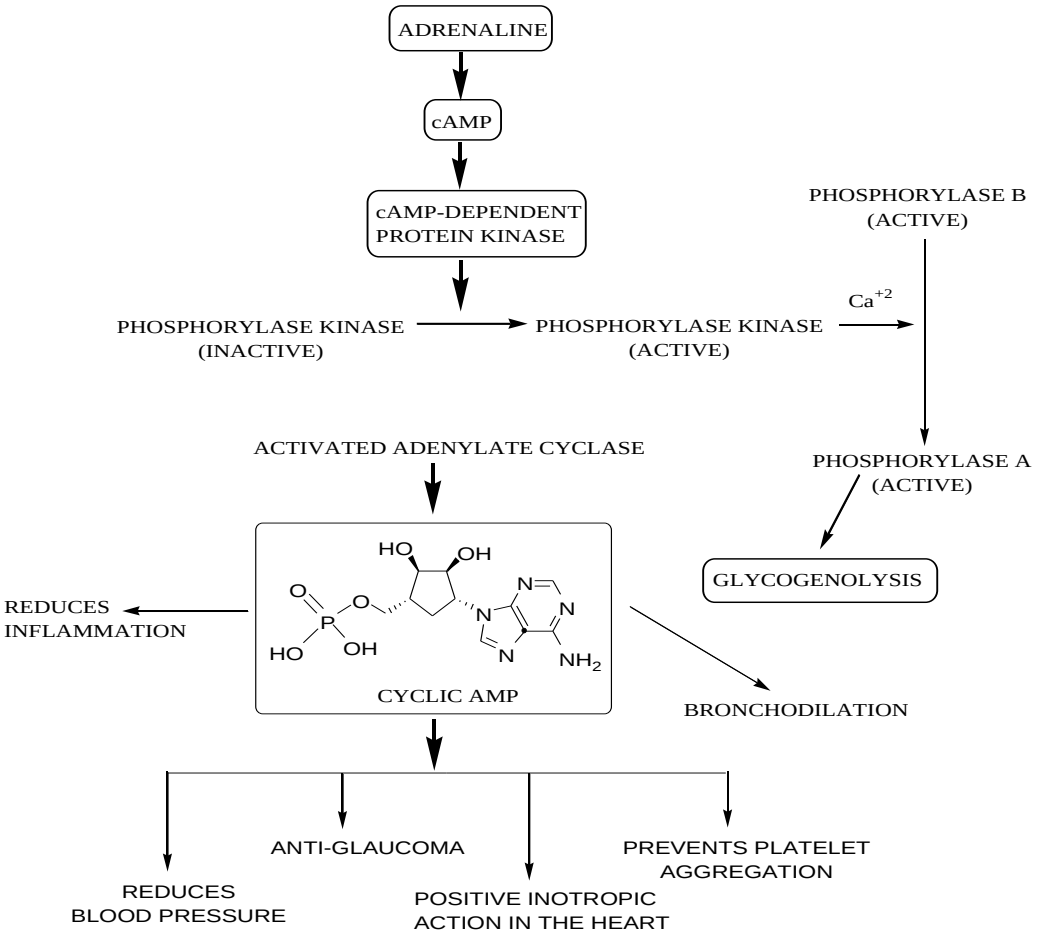
Liver adenylyl cyclase responds more strongly to glucagon, and muscle adenylyl cyclase responds more strongly to adrenaline.

Phosphodiesterases decompose cAMP into AMP.

Function: cAMP is most important second messenger, utilized for intracellular signal transduction, such as transferring the effects of hormones like glucagon and adrenaline, which cannot pass through the cell membrane.

It also regulates the activation of protein kinases and controls the effects of adrenaline and glucagon.

It also regulates the passage of Ca^{2+} through ion channels. cAMP and its associated kinases function in several biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism by activating protein kinase.



1.3.3 Guanosine Triphosphate (GTP):

- Guanosine-5'-triphosphate (GTP) is a purine nucleoside triphosphate. It can act as a substrate for both the synthesis of RNA during the transcription process and of DNA during DNA replication.
- It also has the role of a source of energy or an activator of substrates in metabolic reactions, like that of ATP, but more specific. It is used as a source of energy for protein synthesis and gluconeogenesis.
- GTP is essential to signal transduction, in particular with G-proteins, in second-messenger mechanisms where it is converted to **Guanosine diphosphate** (**GDP**) through the action of GTPases.

I. Uses:

A. Energy Transfer:

GTP is involved in energy transfer within the cell. For instance, a GTP molecule is generated by one of the enzymes in the citric acid cycle.

This is tantamount to the generation of one molecule of ATP, since GTP is readily converted to ATP with nucleoside-diphosphate kinase (NDK).

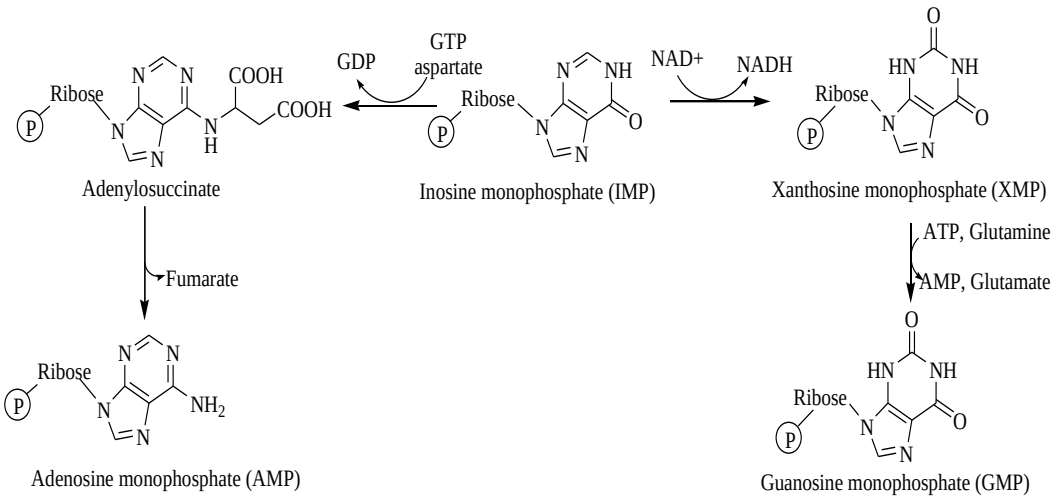
B. Genetic Translation:

During the elongation stage of translation, GTP is used as an energy source for the binding of a new amino- bound tRNA to the A site of the ribosome.

C. Mitochondrial *Function:*

The translocation of proteins into the mitochondrial matrix involves the interactions of both GTP and ATP.

D. Synthesis of AMP and GMP from IMP:



1.4 Cyclic Guanosine Monophosphate:

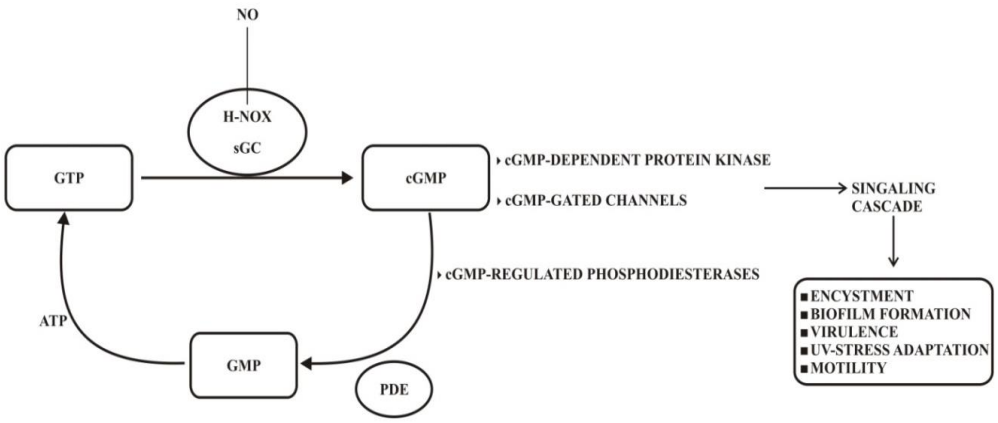
Cyclic guanosine monophosphate (cGMP) is a cyclic nucleotide derived from guanosine triphosphate (GTP).

cGMP acts as a second messenger much like cyclic AMP.

Its most likely mechanism of action is activation of intracellular protein kinases in response to the binding of membrane-impermeable peptide hormones to the external cell surface.

Synthesis: Guanylate cyclase (GC) catalyzes cGMP synthesis.

This enzyme converts GTP to cGMP.



Schematic representation of synthesis, degradation, and function of cGMP. The three targets of cGMP molecules are (i) cGMP dependent protein kinases, (ii) cGMP gated ion channels and (iii) cGMP-dependent phosphodiesterases.

While phosphodiesterases are involved in the degradation of cGMP to GMP, the protein kinases and activation of ion channels are subsequently involved in various bacterial signaling pathways.

GTP: Guanosine 5′-triphosphate;

sGC: soluble guanylate cyclase;

NO: nitric oxide;

H-NOX: Heme-Nitric oxide/Oxygen domain;

cGMP: cyclic guanosine 3′, 5′-monophosphate;

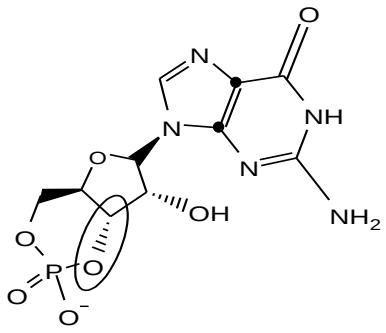
PDE: Phosphodiesterase;

GMP: guanosine 3′, 5′- monophosphate;

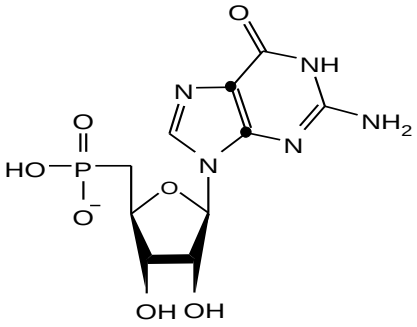
ATP: adenosine 5′-triphosphate.

1.4.1 Effects:

- cGMP is a common regulator of ion channel conductance, glycogenolysis, and cellular apoptosis. It also relaxes smooth muscle tissues. In blood vessels, relaxation of vascular smooth muscles leads to vasodilation and increased blood flow.
- cGMP is a secondary messenger in phototransduction in the eye.
- cGMP is involved in the regulation of some protein-dependent kinases.



Cyclic GMP



GMP

1.5 Chapter at a Glance:

Term	Description
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
ΔG	Free energy
NADPH	Nicotinamide adenine dinucleotide phosphate
$FADH_2$	Flavin adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide

1.6 Exercises:

1.6.1 Multiple Choice Questions:

1. 2, 4-Dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was used as a weight-loss agent in the 1930s. Reports of fatal overdoses led to its discontinuation in 1939. Which of the following would most likely be true concerning individuals taking 2, 4-DNP?

   (a) ATP levels in the mitochondria are greater than normal.
   (b) Body temperature is elevated as a result of hyper metabolism.
   (c) Cyanide has no effect on electron flow
   (d) The proton gradient across the inner mitochondrial membrane is greater than normal.

2. Which of the following has the strongest tendency to gain electrons?

   (a) Coenzyme Q
   (b) Cytochrome c
   (c) Flavin adenine dinucleotide
   (d) Oxygen

3. If enthalpy change for a reaction is zero, then ΔG° equals to

   (a) -TΔS° (b) TΔS°
   (c) -ΔH° (d) ΔH°

4. ΔG° is defined as the

   (a) Residual energy present in the reactants at equilibrium
   (b) Residual energy present in the products at equilibrium
   (c) Difference in the residual energy of reactants and products at equilibrium
   (d) Energy required in converting one mole of reactants to one mole of products

5. for a reaction if ΔG° is positive, then

   (a) The products will be favored
   (b) The reactants will be favored
   (c) The concentration of the reactants and products will be equal
   (d) All of the reactant will be converted to products

6. Unfolding of regular secondary protein structure causes

   (a) Large decrease in the entropy of the protein
   (b) Little increase in the entropy of protein
   (c) No change in the entropy of the protein
   (d) Large increase in the entropy of the protein

7. The study of energy relationships and conversions in biological systems is called as

   (a) Biophysics (b) Biotechnology
   (c) Bioenergetics (d) Microbiology

8. What does first law of thermodynamics state?

   (a) Energy can neither be destroyed nor created
   (b) Energy cannot be 100 percent efficiently transformed from one type to another
   (c) All living organisms are composed of cells
   (d) Input of heat energy increases the rate of movement of atoms and molecules

9. Which of the following statements is false?

   (a) The reaction tends to go in the forward direction if ?G is large and positive
   (b) The reaction tends to move in the backward direction if ?G is large and negative
   (c) The system is at equilibrium if ?G = 0
   (d) The reaction tends to move in the backward direction if ?G is large and positive

10. The free energy of hydrolysis of a compound must be at least _____, in order for it to be considered a "high-energy" compound.

(a) -3 kcal/mol (b) -5 kcal/mol
(c) -7 kcal/mol (d) -9 kcal/mol

1.6.2 Short Answer Questions:

a. Explain why and how the malate-aspartate shuttle moves nicotinamide adenine dinucleotide reducing equivalents from the cytosol to the mitochondrial matrix.
b. Carbon monoxide (CO) binds to and inhibits Complex IV of the electron transport chain. What effect, if any, should this respiratory inhibitor have on phosphorylation of adenosine diphosphate (ADP) to ATP?
c. Give the biological significances of ATP and cyclic AMP

1.6.3 Long Answer Questions:

1. Z. Give the classification of biomolecules.
2. Z. Give the chemical nature and biological role of carbohydrates.
3. Z. Give the detailed account of lipids with their biological role.
4. Z. Give the detailed account of free energy.
5. Z. What are endergonic and exergonic reaction

Answer key MCQs

(1) – (b), (2) – (d), (3) – (a), (4) – (d), (5) – (b), (6) – (d), (7) – (c), (8) – (c), (9) – (d), (10) – (c).

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Chapter 2

Biomolecules

2.1 Introduction:

There is a wide diversity in living organisms in our biosphere. Now a question that arises in our minds is: Are all living organisms made of the same chemicals, i.e., elements and compounds? You have learnt in chemistry how elemental analysis is performed. If we perform such an analysis on a plant tissue, animal tissue or a microbial paste, we obtain a list of elements like carbon, hydrogen, oxygen and several others and their respective content per unit mass of a living tissue. If the same analysis is performed on a piece of earth's crust as an example of non-living matter, we obtain a similar list. What are the differences between the two lists? In absolute terms, no such differences could be made out. All the elements present in a sample of earth's crust are also present in a sample of living tissue. However, a closer examination reveals that the relative abundance of carbon and hydrogen with respect to other elements is higher in any living organism than in earth's crust (Table 2.1).

2.1.1 How to Analyse Chemical Composition?

We can continue asking in the same way, what types of organic compounds are found in living organisms? How does one go about finding the answer? To get an answer, one has to perform a chemical analysis. We can take any living tissue (a vegetable or a piece of liver, etc.) and grind it in trichloroacetic acid (Cl3CCOOH) using a mortar and a pestle. We obtain thick slurry. If we were to strain this through a cheesecloth or cotton we would obtain two fractions. One is called the filtrate or more technically, the acid-soluble pool, and the second, the retentate or the acid-insoluble fraction. Scientists have found thousands of organic compounds in the acid-soluble pool.

In higher classes you will learn about how to analyses a living tissue sample and identify a particular organic compound. It will suffice to say here that one extracts the compounds, then subjects the extract to various separation techniques till one has separated a compound from all other compounds. In other words, one isolates and purifies a compound. Analytical techniques, when applied to the compound give us an idea of the molecular formula and the probable structure of the compound. All the carbon compounds that we get from living tissues can be called 'biomolecules'. However, living organisms have also got inorganic elements and compounds in them. How do we know this? A slightly different but destructive experiment has to be done. One weighs a small amount of a living tissue (say a leaf or liver and this is called wet weight) and dry it. All the water evaporates. The remaining material gives dry weight. Now if the tissue is fully burnt, all the carbon compounds are oxidised to gaseous form (CO_2, water vapour) and are removed. What is remaining is called 'ash'. This ash contains inorganic elements (like calcium, magnesium etc). Inorganic compounds like sulphate, phosphate, etc., are also seen in the acid-soluble fraction.

Therefore elemental analysis gives elemental composition of living tissues in the form of hydrogen, oxygen, chlorine, carbon etc. while analysis for compounds gives an idea of the kind of organic and inorganic constituents (Table 1.2) present in living tissues.

From a chemistry point of view, one can identify functional groups like aldehydes, ketones, aromatic compounds, etc. But from a biological point of view, we shall classify them into amino acids, nucleotide bases, fatty acids etc.

Table 2.1: A Comparison of Elements Present in Non-living and Living Matter*

Element	% Weight of	
	Earth's crust	Human body
Hydrogen (H)	0.14	0.5
Carbon (C)	0.03	18.5
Oxygen (O)	46.6	65.0
Nitrogen (N)	very little	3.3
Sulphur (S)	0.03	0.3
Sodium (Na)	2.8	0.2
Calcium (Ca)	3.6	1.5
Magnesium (Mg)	2.1	0.1
Silicon (Si)	27.7	Negligible

* Adapted from CNR Rao, Understanding Chemistry, Universities Press, and Hyderabad.

Table 2.2: A List of Representative Inorganic Constituents of Living Tissues

Component	Formula
Sodium	Na^+
Potassium	K^+
Calcium	Ca^{++}
Magnesium	Mg^{++}
Water	H_2O
Compounds	NaCl, $CaCO_3$, PO^{3+}, SO^{2-}

Amino acids are organic compounds containing an amino group and an acidic group as substituents on the same carbon i.e., the α-carbon. Hence, they are called α -amino acids. They are substituted methane's. There are four substituent groups occupying the four valency positions. These are hydrogen, carboxyl group, amino group and a variable group designated as R group. Based on the nature of R group there are many amino acids. However, those which occur in proteins are only of twenty types. The R group in these proteinaceous amino acids could be a hydrogen (the amino acid is called glycine), a methyl group (alanine), hydroxy methyl (serine), etc.

The chemical and physical properties of amino acids are essentially of the amino, carboxyl and the R functional groups. Based on number of amino and carboxyl groups, there are acidic (e.g., glutamic acid), basic (lysine) and neutral (valine) amino acids. Similarly, there are aromatic amino acids (tyrosine, phenylalanine, and tryptophan). A particular property of amino acids is the ionizable nature of $–NH_2$ and –COOH groups. Hence in solutions of different PHS, the structure of amino acids changes.

2.1.2 Biomolecules Definition:

a. Biomolecules are molecules that occur naturally in living organisms. Biomolecules include macromolecules like proteins, carbohydrates, lipids and nucleic acids.
b. It also includes small molecules like primary and secondary metabolites and natural products.
c. Biomolecules consists mainly of carbon and hydrogen with nitrogen, oxygen, sulphur, and phosphorus.
d. Biomolecules are very large molecules of many atoms, which are covalently bound together.

2.1.3 Classes of Biomolecules:

a) There are four major classes of biomolecules:

i. Carbohydrates
ii. Lipids
iii. Proteins
iv. Nucleic acids

2.1.4 Carbohydrates:

a. Carbohydrates are good source of energy. Carbohydrates (polysaccharides) are long chains of sugars. Monosaccharides are simple sugars that are composed of 3-7 carbon atoms.
b. They have a free aldehyde or ketone group, which acts as reducing agents and are known as reducing sugars. Disaccharides are made of two monosaccharides. The bonds shared between two monosaccharides are the glycosidic bonds.
c. Monosaccharides and disaccharides are sweet, crystalline and water soluble substances. Polysaccharides are polymers of monosaccharides. They are un-sweet and complex carbohydrates. They are insoluble in water and are not in crystalline form.

d. **Example:** glucose, fructose, sucrose, maltose, starch, cellulose etc.

I. Lipids:

a. Lipids are composed of long hydrocarbon chains. Lipid molecules hold a large amount of energy and are energy storage molecules. Lipids are generally esters of fatty acids and are building blocks of biological membranes.
b. Most of the lipids have a polar head and non-polar tail. Fatty acids can be unsaturated and saturated fatty acids.
c. Lipids present in biological membranes are of three classes based on the type of hydrophilic head present:

   i. Glycolipids are lipids whose head contains oligosaccharides with 1-15 saccharide residues.
   ii. Phospholipids contain a positively charged head which are linked to the negatively charged phosphate groups.
   iii. Sterols, whose head contain a steroid ring. Example steroid.

Example of lipids: oils, fats, phospholipids, glycolipids, etc

II. Nucleic Acids:

A. Nucleic acids are organic compounds with heterocyclic rings. Nucleic acids are made of polymer of nucleotides. Nucleotides consist of nitrogenous base, a pentose sugar and a phosphate group. A nucleoside is made of nitrogenous base attached to a pentose sugar. The nitrogenous bases are adenine, guanine, tyramine, cytosine and uracil. Polymerized nucleotides form DNA and RNA which are genetic material.

III. Proteins:

a. Proteins are heteropolymers of stings of amino acids. Amino acids are joined together by the peptide bond which is formed in between the carboxyl group and amino group of successive amino acids. Proteins are formed from 20 different amino acids, depending on the number of amino acids and the sequence of amino acids.
b. There are four levels of protein structure:

(i) Primary Structure of Protein: Here protein exist as long chain of amino acids arranged in a particular sequence. They are non-functional proteins.
(ii) Secondary Structure of Protein: The long chain of proteins are folded and arranged in a helix shape, where the amino acids interact by the formation of hydrogen bonds. This structure is called the pleated sheet. Example: silk fibres.
(iii) Tertiary Structure of Protein: Long polypeptide chains become more stabilizes by folding and coiling, by the formation of ionic or hydrophobic bonds or disulphide bridges, these results in the tertiary structure of protein.
(iv) Quaternary Structure of Protein: When a protein is an assembly of more than one polypeptide or subunits of its own, this is said to be the quaternary structure of protein. Example: Haemoglobin, insulin.

2.1.4 Functions of Biomolecules:

a. **Carbohydrates** provide the body with source of fuel and energy, it aids in proper functioning of our brain, heart and nervous, digestive and immune system. Deficiency of carbohydrates in the diet causes fatigue, poor mental function.
b. Each **protein** in the body has specific functions, some proteins provide structural support, help in body movement, and also defense against germs and infections. Proteins can be antibodies, hormonal, enzymes and contractile proteins.
c. **Lipids**, the primary purpose of lipids in body are energy storage. Structural membranes are composed of lipids which form a barrier and controls flow of material in and out of the cell. Lipid hormones, like sterols, help in mediating communication between cells.

Nucleic Acids are the **DNA** and **RNA**; they carry genetic information in the cell. They also help in synthesis of proteins, through the process of translation and transcription.

2.2 Carbohydrates:

a. Carbohydrate is an organic compound, it comprises of only oxygen, carbon and hydrogen.
b. The oxygen: hydrogen ratio is usually is 2:1.
c. The empirical formula being $\mathbf{C_n (H_2O)_n}$.
d. Carbohydrates are hydrates of carbon; technically they are polyhydroxy aldehydes and ketones.
e. Carbohydrates are also known as saccharides, the word saccharide comes from Greek word **sakkron** which means sugar.

2.2.1 Classification and Nomenclature of Carbohydrates:

A. The carbohydrates are divided into three major classes depending upon whether or not they undergo hydrolysis and if they do, on the number of products formed.

i. Monosaccharides: The monosaccharides are polyhydroxy aldehydes or polyhydroxy ketones which cannot be decomposed by hydrolysis to give simpler carbohydrates. E.g. Glucose, fructose, Galactose etc.

ii. **Oligosaccharides:** The oligosaccharides (Oligo: few) are carbohydrates which yield a definite number (2-9) of monosaccharide molecules on hydrolysis.

a. Disaccharides: Which yield two monosaccharides molecules on hydrolysis. Which have molecular formula is $C_{12}H_{22}O_{11}$.e.g. Sucrose, maltose etc.

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{H^+} \underset{\text{Fructose}}{C_6H_{12}O_6} + \underset{\text{Glucose}}{C_6H_{12}O_6} \qquad \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{H+} \underset{\text{Glucose}}{2C_6H_{12}O_6}$$

b. Trisaccharides - Which yield three monosaccharides molecules on hydrolysis and have molecular formula is $C_{18}H_{32}O_{16}$.

$$C_{18}H_{32}O_{16} + H_2O \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_{12}O_6 + C_6H_{12}O_6$$

Raffinose — Fructose — Glucose — Galactose

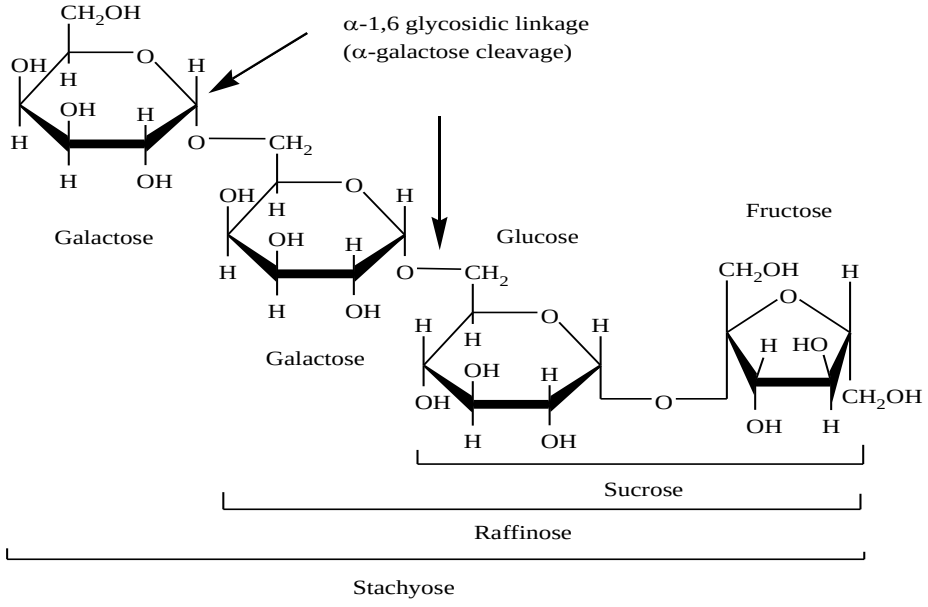
c. Tetrasaccharides - Which yield four monosaccharides molecules on hydrolysis and have molecular formula is $C_{22}H_{42}O_{21}$. e.g: Stachyose [gal (α1→6) gal (α1→6) glu (α1↔2β) fru]

iii. Polysaccharides: The carbohydrates which have higher molecular weight, which yield many monosaccharide molecules on hydrolysis. e.g: Starch, glycogen, Dextrin, Cellulose etc.

$$(C_6H_{10}O_5)_n + H_2O \xrightarrow{H^+} nC_6H_{12}O_6$$

Starch or Cellulose — Glucose

In general monosaccharides and oligosaccharides are crystalline solids, soluble in water and sweet to taste, they are collectively known as sugars, the polysaccharides on the other hand are amorphous, insoluble in water and tasteless, they are called non-sugars.

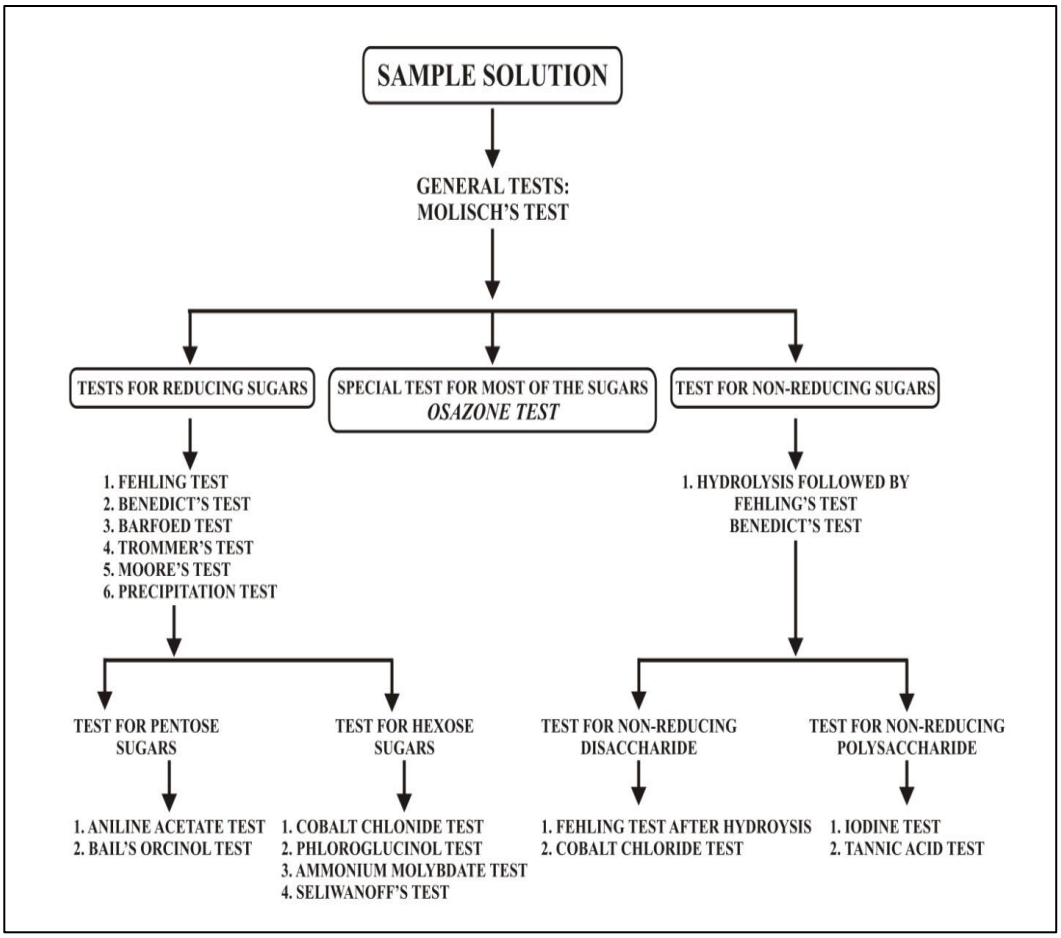


Different between Monosaccharaides, Oligosaccharides and Polysaccharides

Character	Monosaccharides	Oligosaccharides	Polysaccharides
No. of sugar molecules	1	2-9	More than 9
Glycoside bond	Absent	Present	Present
Molecular Weight	Low	Moderate	High
Taste	Sweet	Minimally sweet Taste	No taste
Solubility	Soluble	Soluble	Insoluble
Nature	Always reducing sugar	May or may not be	Always non-reducing sugar

Example Glucose, fructose, Sucrose, Maltose Starch, Glycogen, Galactose Dextrin, Cellulose

Test for carbohydrate:



2.2.2 Properties of Carbohydrates:

a. General Properties of Carbohydrates:

Carbohydrates act as energy reserves, also stores fuels, and metabolic intermediates.

- Ribose and deoxyribose sugars forms the structural frame of the genetic material, RNA and DNA.
- Polysaccharides like cellulose are the structural elements in the cell walls of bacteria and plants.
- Carbohydrates are linked to proteins and lipids that play important roles in cell interactions.
- Carbohydrates are organic compounds; they are aldehydes or ketones with many hydroxyl groups.

b. Physical Properties of Carbohydrates:

- Steroisomerism: Compound shaving same structural formula but they differ in spatial configuration.
  Example: Glucose has two isomers with respect to penultimate carbon atom. They are D-glucose and L-glucose.
- Optical Activity: It is the rotation of plane polarized light forming (+) glucose and (-) glucose.
- Diastereo isomers: It the configurational changes with regard to C2, C3, or C4 in glucose.
  Example: Mannose, galactose.
- Annomerism: It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

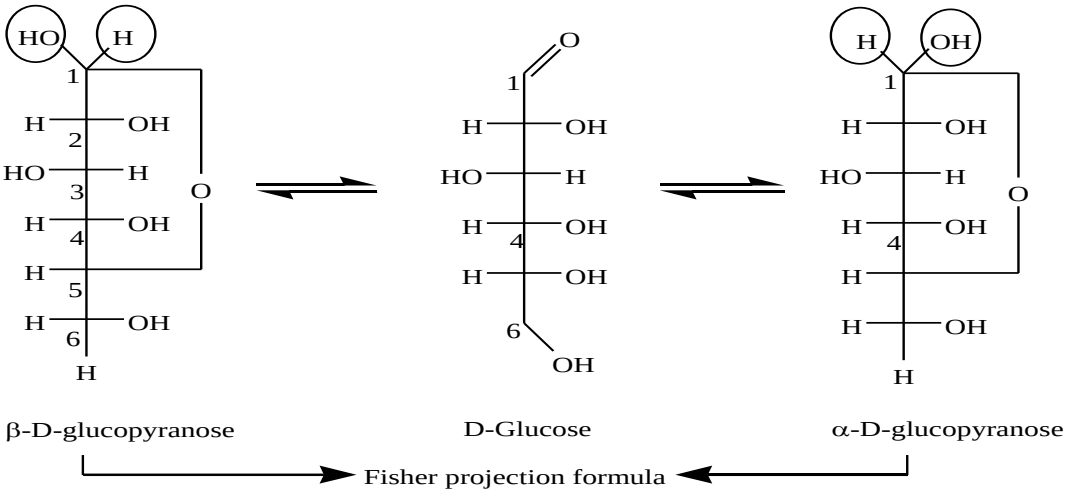
c. Chemical properties of Carbohydrates

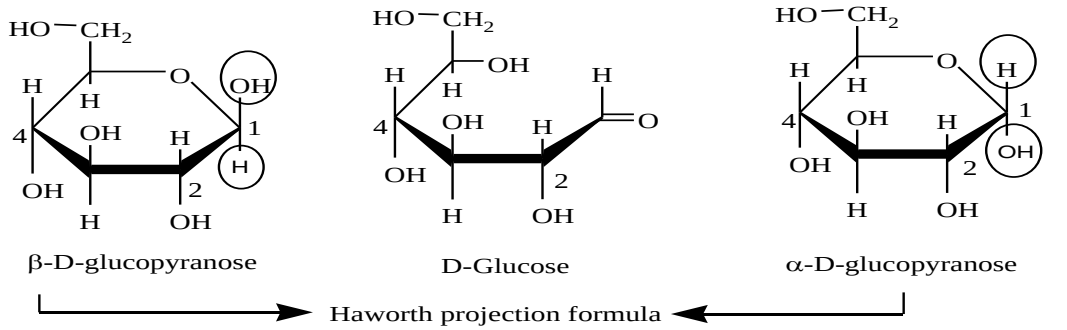
- Osazone formation with phenylhydrazine.
- Benedicts test.
- Oxidation
- Reduction to alcohols

2.2.3 Structure of Carbohydrates:

There are three types of structural representations of carbohydrates:

(i) Open chain structure.
(ii) Hemi-acetal structure.
(iii) Haworth structure.





2.2.4 Functions of Carbohydrates:

- Carbohydrates are chief energy source, in many animals; they are instant source of energy. Glucose is broken down by glycolysis/kreb's cycle to yield ATP.
- Glucose is the source of storage of energy. It is stored as glycogen in animals and starch in plants.
- Stored carbohydrates act as energy source instead of proteins.
- Carbohydrates are intermediates in biosynthesis of fats and proteins.
- Carbohydrates aid in regulation of nerve tissue and are the energy source for brain.
- Carbohydrates get associated with lipids and proteins to form surface antigens, receptor molecules, vitamins and antibiotics.
- They form structural and protective components, like in cell wall of plants and microorganisms.
- In animals they are important constituent of connective tissues.
- They participate in biological transport, cell-cell communication and activation of growth factors.
- Carbohydrates those are rich in fibre content help to prevent constipation.
- Also they help in modulation of immune system.

2.2.5 Example of Carbohydrates:

a. **Monosaccharides:** Glucose, galactose, glycerose, erythrose, ribose, ribulose, fructose.
b. **Oligosaccharides:** Maltose, lactose, sucrose, raffinose, stachyose.
c. **Polysaccharides:** Starch, glycogen, cellulose, pectin, inulin, hyaluonic acid.

Foods rich in carbohydrates are referred to as starchy foods. They are found in legumes, starchy vegetables, whole-grain breads and cereals.

They also occur naturally with vitamins and minerals in foods like milk, fruits, and milk products.

They are also found in refined and processed products like candy, carbonated beverages, and table sugar.

Examples of Polysaccharides

Name of the Polysaccharide	Composition	Occurrence	Functions
Starch	Polymer of glucose containing a straight chain of glucose molecules (amylose) and a branched chain of glucose molecules (amylopectin)	In several plant species as main storage carbohydrate	Storage of reserve food
Glycogen	Polymer of glucose	Animals (equivalent of starch)	Storage of reserve Food
Inulin	Polymer of fructose	In roots and tubers (like Dahlia)	Storage of reserve Food
Cellulose	Polymer of glucose	Plant cell wall	Cell wall matrix
Pectin	Polymer of galactose and its derivatives	Plant cell wall	Cell wall matrix
Hemi cellulose	Polymer of pentoses and sugar acids	Plant cell wall	Cell wall matrix
Lignin	Polymer of glucose	Plant cell wall (dead cells like sclerenchyma)	Cell wall matrix
Chitin	Polymer of glucose	Bodywall of arthropods. In some fungi also	Exoskeleton Impermeable to water
Murein	Polysaccharide cross linked with amino acids	Cell wall of prokaryotic cells	Structural protection

Name of the Polysaccharide	Composition	Occurrence	Functions
Hyaluronic acid	Polymer of sugar acids	Connective tissue matrix, Outer coat of mammalian Eggs	Ground substance, Protection
Heparin	Closely related to chrondroitin	Connective tissue cells	Anticoagulant
Gums and mucilages	Polymers of sugars and sugar acids	Gums - bark or trees. Mucilages - flower	Retain water in dry Seasons

2.3 Lipids:

a. Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by nonpolar solvents, because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles or on albumin.
b. Lipids are a major source of energy for the body, and they provide the hydrophobic barrier.
c. Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.

2.3.1 General Characters of Lipids:

a. Lipids are relatively insoluble in water.
b. They are soluble in non-polar solvents, like ether, chloroform, and methanol.
c. Lipids have high energy content and are metabolized to release calories.
d. Lipids also act as electrical insulators, they insulate nerve axons.
e. Fats contain saturated fatty acids; they are solid at room temperatures. Example, animal fats.
f. Plant fats are unsaturated and are liquid at room temperatures.
g. Pure fats are colorless, they have extremely bland taste.
h. The fats are sparingly soluble in water and hence are described are hydrophobic substances.
i. They are freely soluble in organic solvents like ether, acetone and benzene.
j. The melting point of fats depends on the length of the chain of the constituent fatty acid and the degree of unsaturation.
k. Geometric isomerism, the presence of double bond in the unsaturated fatty acid of the lipid molecule produces geometric or cis-trans isomerism.
l. Fats have insulating capacity; they are bad conductors of heat.

m. Emulsification is the process by which a lipid mass is converted to a number of small lipid droplets. The process of emulsification happens before the fats can be absorbed by the intestinal walls.
n. The fats are hydrolyzed by the enzyme lipases to yield fatty acids and glycerol.
o. The hydrolysis of fats by alkali is called saponification. This reaction results in the formation of glycerol and salts of fatty acids called soaps.
p. Hydrolytic rancidity is caused by the growth of microorganisms which secrete enzymes like lipases. These split fats into glycerol and free fatty acids.

2.3.2 Classification of Lipids:

I. Simple Lipids:

Esters of fatty acids with various alcohols.

a. Fats: Esters of fatty acids with glycerol. **Oils** are fats in the liquid state.
b. Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.

II. Complex Lipids:

Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

a. Phospholipids: Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, eg, in **glycerophospholipids** the alcohol is glycerol and in **sphingophospholipids** the alcohol is sphingosine.
b. Glycolipids (Glycosphingolipids): Lipids containing a fatty acid, sphingosine, and carbohydrate.
c. Other Complex Lipids: Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

III. Precursor and Derived Lipids:

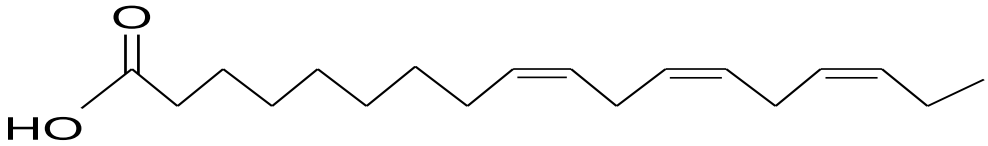
These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins and hormones.

Essential Fatty Acids:

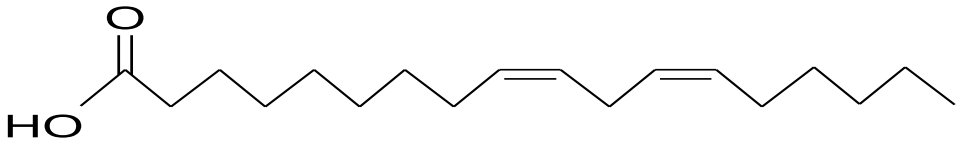
a) Two fatty acids are dietary essentials in humans

i. **Linoleic acid**, which is the precursor of arachidonic acid, the substrate for prostaglandin synthesis.
ii. **α-linolenic** acid is the precursor for growth and development.

Essential fatty acid deficiency can result in a scaly dermatitis, as well as visual and neurologic abnormalities.



Linolenic acid



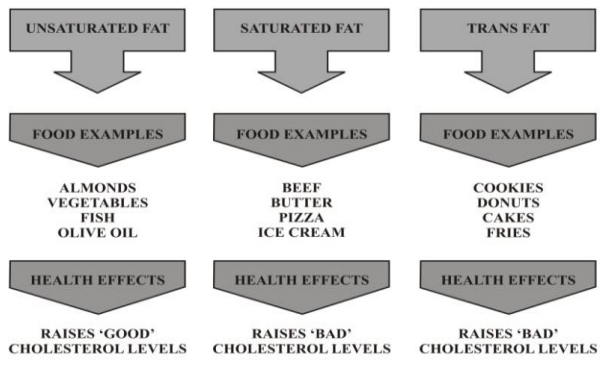
α-Linoleic acid

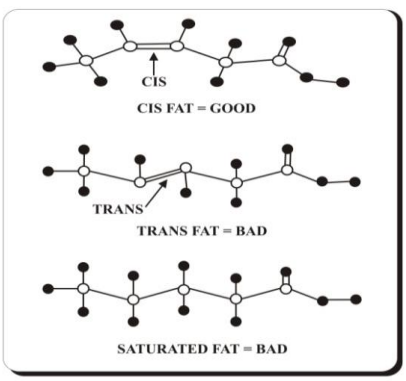
IV. Regulating Blood Cholesterol Levels:

a. Fats and cholesterol cannot dissolve in blood and are consequently packaged with proteins (to form lipoproteins) for transport
   - Low density lipoproteins (LDL) carry cholesterol from the liver to the rest of the body
   - High density lipoproteins (HDL) scavenge excess cholesterol and carry it back to the liver for disposal

a. Hence LDLs raise blood cholesterol levels ('bad') while HDLs lower blood cholesterol levels ('good')
b. High intakes of certain types of fats will differentially affect cholesterol levels in the blood
   - Saturated fats increase LDL levels within the body, raising blood cholesterol levels
   - Trans fats increase LDL levels **and** decrease HDL levels within the body, significantly raising blood cholesterol levels

Unsaturated (cis) fats increase HDL levels within the body, lowering blood cholesterol levels





2.3.3 Lipid Health Claims:

a. There are two main health claims made about lipids in the diet:

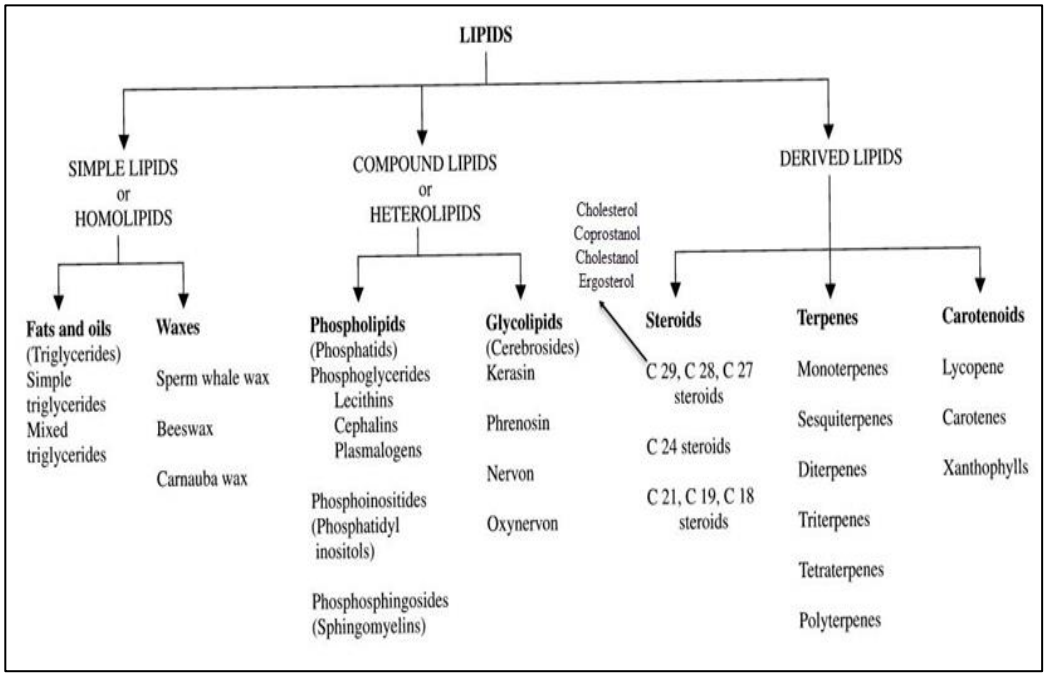
- Diets rich in **saturated fats** and **trans fats** increase the risk of CHD
- Diets rich in **monounsaturated** and **polyunsaturated (cis) fats** decrease the risk of CHD

Health Risks of High Cholesterol:

a. High cholesterol levels in the bloodstream lead to the hardening and narrowing of arteries (atherosclerosis)
b. When there are high levels of LDL in the bloodstream, the LDL particles will form deposits in the walls of the arteries
c. The accumulation of fat within the arterial walls leads to the development of plaques which restrict blood flow
d. If coronary arteries become blocked, **Coronary Heart Disease** (**CHD**) will result – this includes heart attacks and strokes

2.3.4 Examples of Lipids:

a. **Fatty acids:** Oleic acid, Linoleic acid, Palmitoleic acid, Arachidonic acid.
b. **Fats and Oils: Animal fats** - Butter, Lard, Human fat, Herring oil.
c. **Plant oils:** Coconut oil, Corn, Palm, Peanut, Sunflower oil.
d. **Waxes:** Spermacti, Beeswax, Carnauba wax.
e. **Phospholipids:** Lecithins, Cephalins, Plasmoalogens, Phosphatidyl inositols, Sphingomyelins.
f. **Glycolipids:** Kerasin, Phrenosin, Nervon, Oxynervon.
g. **Steroids:** Cholesterol.
h. **Terpenes:** Monoterpenes, Sesquiterpenes, Diterpenes, Triterpenes.
i. **Carotenoids:** Lycopene, Carotenes, Xanthophylls.



2.3.5 Biological Role of Lipids:

a. Food Material: Lipids provide food, highly rich in calorific value. One gram lipid produces 9.3 kilocalories of heat.
b. Food Reserve: Lipids provide are insoluble in aqueous solutions and hence can be stored readily in the body as a food reserve.
c. Structural Component: Lipids are an important constituent of the cell membrane.
d. Heat Insulation: The fats are characterized for their high insulating capacity. Great quantities of fat are deposited in the subcutaneous layers in aquatic mammals such as whale and in animals living in cold climates.
e. Fatty Acid Absorption: Phospholipids play an important role in the absorption and transportation of fatty acids.
f. Hormone Synthesis: The sex hormones, adrenocorticoids, cholic acids and also vitamin D are all synthesized from cholesterol, a steroidal lipid.
g. Vitamin Carriers: Lipids act as carriers of natural fat-soluble vitamins such as vitamin A, D and E.
h. Blood Cholesterol Lowering: Chocolates and beef, especially the latter one, were believed to cause many heart diseases as they are rich in saturated fatty acids, which boost cholesterol levels in blood and clog the arterial passage.

But researchers conducted at the University of Texas by Scott Grundy and Andrea Bonanome (1988) suggest that at least one saturated fatty acid stearic acid, a major component of cocoa butter and beef fat, does not raise blood cholesterol level at all.

The researchers placed 11 men on three cholesterol poor liquid diets for three weeks each in random order.

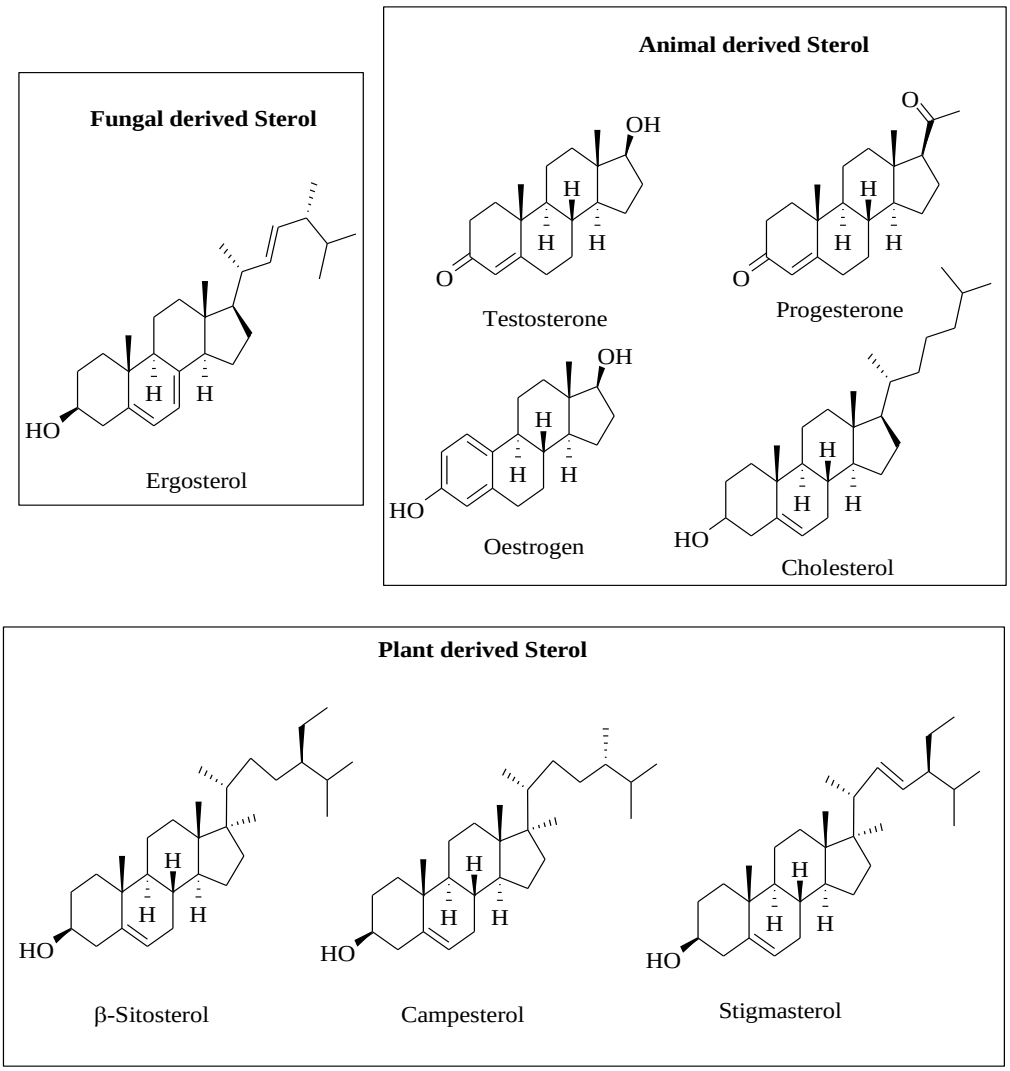
One formula was rich in palmitic acid, a known cholesterol booster; the second in oleic acid; and the third in stearic acid.

When compared with the diet rich in palmitic acid, blood cholesterol levels were 14% lower in subjects put on the stearic acid diet and 10% lower in those on the oleic acid diet.

i. Antibiotic Agent:

Squalamine, a steroid from the blood of sharks, has been shown to be an antibiotic and antifungal agent of intense activity.

This seems to explain why sharks rarely contract infections and almost never get cancer.



2.4 Proteins:

a. Proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues.
b. Proteins are known as building blocks of life.
c. Proteins are the most abundant intracellular macro-molecules. They provide structure, protection to the body of multicellular organism in the form of skin, hair, callus, cartilage, ligaments, muscles, tendons. Proteins regulate and catalyze the body chemistry in the form of hormones, enzymes, immunoglobulin's etc.

2.4.1 General Characteristics of Proteins:

a. Proteins are organic substances; they are made up of nitrogen and also, oxygen, carbon and hydrogen.
b. Proteins are the most important biomolecules; they are the fundamental constituent of the cytoplasm of the cell.
c. Proteins are the structural elements of body tissues.
d. Proteins are made up of amino acids.
e. Proteins give heat and energy to the body and also aid in building and repair.
f. Only small amounts of proteins are stored in the body as they can be used up quickly on demand.
g. Proteins are considered as the bricks, they make up bones, muscles, hair and other parts of the body.
h. Proteins like enzymes are functional elements that take part in metabolic reactions.
i. Antibodies, blood haemoglobin are also made of proteins.
j. Proteins have a molecular weight of 5 to 300 kilo-Daltons.

2.4.2 Physical Properties of Proteins:

a. Proteins are colorless and tasteless.
b. They are homogeneous and crystalline.
c. Proteins vary in shape; they may be simple crystalloid structure to long fibrilar structures.
d. Protein structures are of two distinct patterns - Globular proteins and fibrilar proteins.
e. Globular proteins are spherical in shape and occur in plants. Fibrilar proteins are thread-like, they occur generally in animals.
f. In general proteins have large molecular weights ranging between 5×10^3 and 1×10^6.
g. Due to the huge size, proteins exhibit many colloidal properties.
h. The diffusion rates of proteins are extremely slow.
i. Proteins exhibit Tyndall effect.
j. Proteins tend to change their properties like denaturation. Many a times the process of denaturation is followed by coagulation.
k. Denaturation may be a result of either physical or chemical agents. The physical agents include, shaking, freezing, heating etc. Chemical agents are like X-rays, radioactive and ultrasonic radiations.

l. Proteins like the amino acids exhibit amphoteric property i.e., they can act as Acids and Alkalies.
m. As the proteins are amphoteric in nature, they can form salts with both cations and anions based on the net charge.
n. The solubility of proteins depends upon the pH. Lowest solubility is seen at isoelectric point, the solubility increases with increase in acidity or alkalinity.
o. All the proteins show the plane of polarized light to the left, i.e., **laevorotatory**.

2.4.3 Chemical Properties of Proteins:

a. Proteins when hydrolyzed by acidic agents, like conc.HCl yield amino acids in the form of their hydrochlorides.
b. Proteins when are hydrolyzed with alkaline agents leads to hydrolysis of certain amino acids like arginine, cysteine, serine, etc., also the optical activity of the amino acids is lost.
c. Proteins with reaction with alcohols give its corresponding esters. This process is known as esterification.
d. Amino acid reacts with amines to form amides.
e. When free amino acids or proteins are said to react with mineral acids like HCl, the acid salts are formed.
f. When amino acid in alkaline medium reacts with many acid chlorides, acylation reaction takes place.

Xanthoproteic test: On boiling proteins with conc. HNO_3, yellow color develops due to presence of benzene ring.

Folin's test: This is a specific test for tyrosine amino acid, where blue color develops with phosphomolybdotungstic acid in alkaline solution due to presence of phenol group.

2.4.4 Structure of Proteins:

a. Proteins are constructed by polymerization of only 20 different amino acids into linear chains.
b. Proteins are the polymers of L-a-amino acids. The structure of proteins is rather complex which can be divided into 4 levels of organization.

2.4.4.1 Primary Structure:

- The linear sequence of amino acids forming the backbone of proteins (polypeptides).
- Examples of protein with a primary structure are **Hexosaminidase, Dystrophin.**

2.4.4.2 Secondary Structure:

- The spacial arrangement of protein by twisting of the polypeptide chain.
- Example of protein with a secondary structure is **Myoglobin.**

2.4.4.3 Tertiary Structure:

- The three dimensional structure of a functional protein.
- Number of forces act to hold the polypeptide chain in this final configuration:

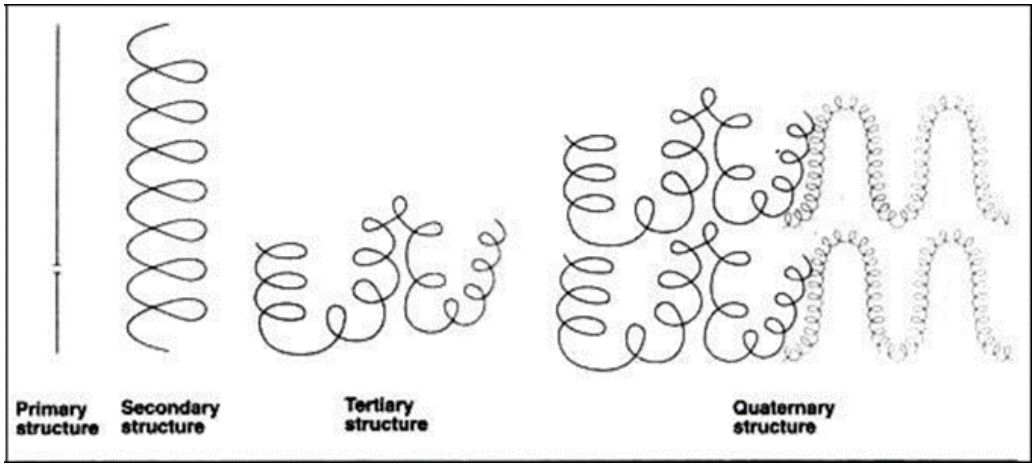
    - Polar/Nonpolar Interactions
    - Hydrogen Bonds
    - Van der Waals Forces
    - Ionic Interactions
    - Disulfide Bonds

    - Examples of protein with a Tertiary structure are **Globular Proteins** (Enzymes) and **Fibrous Proteins**.

2.4.3.4 Quaternary Structure:

- Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spacial arrangement of these subunits is known as quaternary structure.

Examples of protein with a Quaternary structure are **DNA polymerase**, and **ion channels**.



2.4.5 Secondary Structure of Proteins:

a. Shape:

- **Alpha Helix:** Alpha Helix is a right-handed coiled rod-like structure.
- **Beta Pleated Sheet:** Beta sheet is a sheet-like structure.

b. Formation:

- **Alpha Helix:** Hydrogen bonds form within the polypeptide chain in order to create a helical structure.

- **Beta Pleated Sheet:** Beta sheets are formed by linking two or more beta strands by H bonds.

c. Bonds:

- **Alpha Helix:** Alpha helix has n + 4 H-bonding scheme. i.e. Hydrogen bonds form between N-H group of one amino residue with C=O group of another amino acid, which is placed in 4 residues earlier.
- **Beta Pleated Sheet:** Hydrogen bonds are formed in between the neighboring N-H and C=O groups of adjacent peptide chain

d. -R group:

- **Alpha Helix:** -R groups of the amino acids are oriented outside of the helix.
- **Beta Pleated Sheet:** -R groups are directed to both inside and outside of the sheet.

e. Number:

- **Alpha Helix:** This can be a single chain.
- **Beta Pleated Sheet:** This cannot exist as a single beta strand; there are must be two or more.

f. Type:

- **Alpha Helix:** This has only one type.
- Beta Pleated Sheet: This can be parallel, anti-parallel or mixed.

g. Qualities:

- **Alpha Helix:** 100^{o} rotation, 3.6 residues per turn and 1.5 A^{o} rise from one alpha carbon to the second.
- **Beta Pleated Sheet:** 3.5 A^{o} rise between residues.

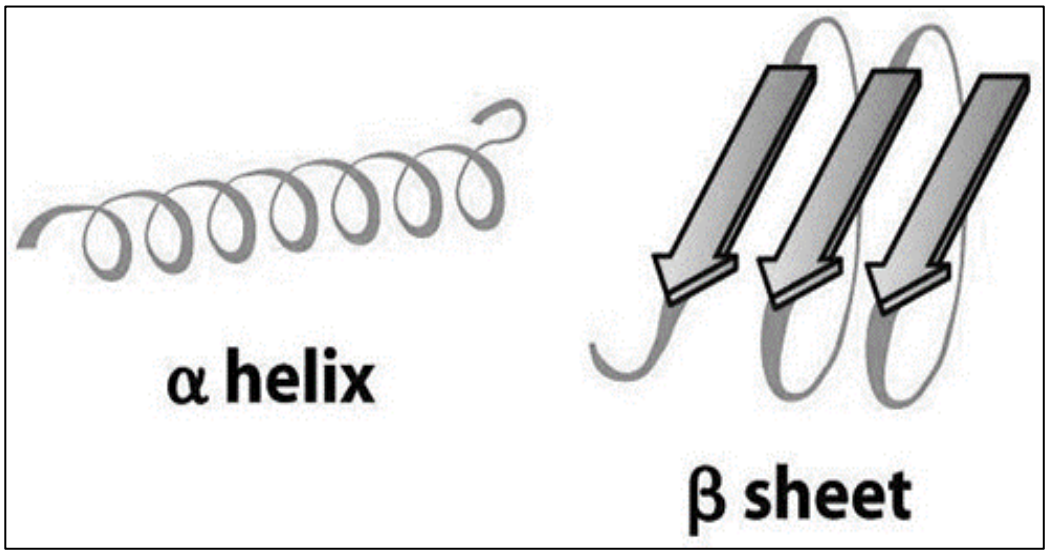
h. Amino Acid:

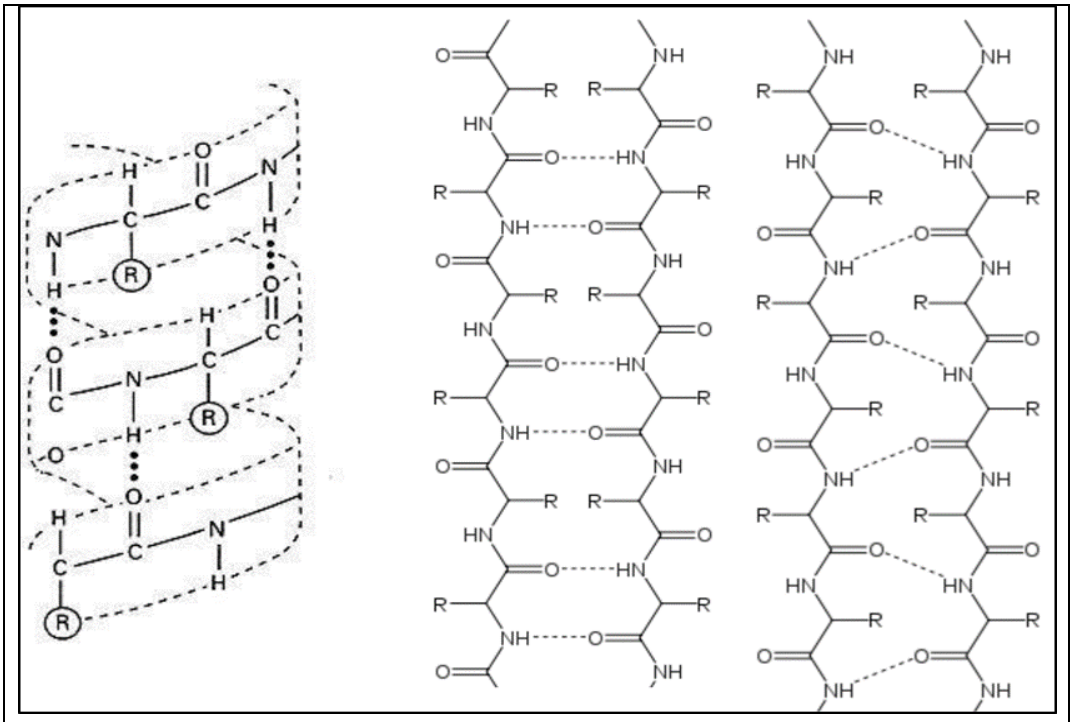
- **Alpha Helix:** Alpha helix prefers the amino acid side chains, which can cover and protect the backbone H-bonds in the core of the helix.
- **Beta Pleated Sheet:** The extended structure leaves the maximum space free for the amino acid side chains. Therefore, amino acids with large bulky side chains prefer beta sheet structure.

i. Preference:

- **Alpha Helix:** Alpha helix prefers Ala, Leu, Met, Phe, Glu, Gln, His, Lys, Arg amino acids.

- **Beta Pleated Sheet:** Beta sheet prefers Tyr, Trp, (Phe, Met), Ile, Val, Thr, Cys.





Alpha Helix Secondary Structure of Proteins	**Beta pleate Antiparallel**: when the adjacent Polypeptide chains run in opposite direction	**Beta pleate Parallel:** Adjacent polypeptide

2.4.6 Protein Classification:

Classification of Proteins Based on Shape:

i. Globular or Corpuscular Proteins:

a. Globular proteins have axial ratio less than 10 but not below 3 or 4.
b. They are compactly folded and coiled and possess a relatively spherical or ovoid shape.
c. They are usually soluble in water and in aqueous media.

Example: Insulin, plasma albumin, globulin enzymes.

Axial ratio, for any structure or shape with two or more axes, is the ratio of the length (or magnitude) of those axes to each other - the longer axis divided by the shorter.

In chemistry or materials science, the axial ratio (symbol P) is used to describe rigid rod-like molecules.

It is defined as the length of the rod divided by the rod diameter.

ii. Fibrous or Fibrillar Proteins:

a. These proteins have axial ratio more than 10, hence, they resemble long ribbons or fibres in shape.
b. They are mostly found in animals, and are not soluble in water or in solution of dilute acids.
c. Fibrous proteins aid in protection and structural support.
d. **Example:** Collagen, Keratin, Elastins, Fibroin

2.4.7 Classification of Proteins Based on Composition and Solubility:

I. Simple Proteins or Holoproteins:

- These proteins are made of only one type of amino acid, as structural component, on decomposition with acids, they liberate constituent amino acids. They are mostly globular type of proteins except for scleroproteins, which are fibrous in nature.
- Simple proteins are further classified based on their solubility.

a. Protamines and Histones:

- These proteins occur only in animals and are basic proteins.
- They possess simple structure and low molecular, are water soluble and are not coagulated by heat.
- They are strongly basic in character due to the high content of lysine, arginine.
- **Example:** Protamines - salmine, clupine, cyprinine; Histones - nucleoshistones, globin.

b. Albumins:

- They are widely distributed in nature, mostly seen in seeds.
- They are soluble in water and dilute solutions of acids, bases and salts.
- **Example:** Leucosine, legumeline, serum albumin.

c. Globulins:

- They are of two types, pseudoglobulins which are soluble in water,
- Other is euglobulins which are insoluble in water.
- They are coagulated by heat.
- **Example:** Pseudoglobulin, serum globulin, glycinine. etc.

d. Scleroproteins or Albuminoids:

- These occur mostly in animals and are commonly known as animal skeleton proteins.
- They are insoluble in water, and in dilute solution of acids, based and salts.

II. Conjugated or Complex Proteins or Heteroproteins:

- These are proteins that are made of amino acids and other organic compounds. The non-amino acid group is termed as prosthetic group.
- Complex proteins are further classified based on the type of prosthetic group present.

a. Metalloproteins:

- These are proteins linked with various metals.
- Example: casein, collagen, ceruloplasmin etc.

b. Chromoproteins:

- These are proteins that are coupled with a colored pigment.
- Example: Myoglubin, hemocyanin, cytochromes, flavoproteins, etc.

C. Glycoproteins and Mucoproteins:

- These proteins contain carbohydrates as the prosthetic group.
- Example: Glycoproteins - egg albumin, serum globulins, serum albumins; Mucoproteins - Ovomucoid, mucin etc.

d. Phosphoproteins:

- These proteins are linked with phosphoric acid.

- Example: casein.

e. Lipoproteins:

- Proteins forming complexes with lipids are lipoproteins.
- Example: lipovitellin, lipoproteins of blood.

f. Nucleoproteins:

- These are compounds containing nucleic acids and proteins.
- Example: Nucleoproteins, nucleohistones, nuclein.

g. Derived Proteins:

- These are proteins that are derived from the action of heat, enzyme or chemical reagents.
- Derived proteins are of two types, primarily derived proteins and secondary derived proteins.

Primary Derived Proteins:

- Derivatives of proteins, in which the size of the protein molecule is not altered materially.
- Primary derived proteins are classified into three types - **Proteans**, **Infraproteins** and **Coagulated proteins**.
- **Example:** edestan, coagulated egg-white.

Secondary Derived Proteins:

- While in secondary derived proteins, hydrolysis occurs, as a result the molecules are smaller than the original proteins.

They are further classified into 3 types - Proteoses, Peptones and Polypeptides

EGG PROTEINS COMPOSITION OF EGG WHITE

Protein		**Percentage**
Total protein		10-11% (on wet basis); 82.8% (on dry basis)
Ovalbumin		70% of total proteins
Conalbumin		9%
Ovomucoid		13%
	Lysozyme (G_1)	2.6%
Globulins	Lysozyme (G_2	7%
	Lysozyme (G_3)	7%

Protein	Percentage
Mucin	2%
Avidin	0.06%

2.4.8 Classification of Proteins on Biological Function:

i. Enzymic Proteins:

a. They are the most varied and highly specialized proteins with catalytic activity. Enzymes catalyze a variety of reactions.
b. **Example:** Urease, catalase, cytochrome C, etc.

ii. Structural Proteins:

a. These proteins aid in strengthening or protecting biological structures.
b. **Example:** Collagen, elastin, keratin, etc.

iii. Transport or Carrier Proteins:

a. These proteins help in transport of ions or molecules in the body.
b. **Example:** Myoglobin, hemoglobin, etc.

iv. Nutrient and Storage Proteins

a. These proteins provide nutrition to growing embryos and store ions.

v. Contractile or Motile Proteins

a. These proteins function in the contractile system.
b. **Example:** Actin, myosin, tubulin, etc.

vi. Defense Proteins

a. These proteins defend against other organisms.
b. **Example:** Antibodies, Fibrinogen, thrombin.

vii. Regulatory Proteins

a. They regulate cellular or metabolic activities.
b. **Example:** Insulin, G proteins, etc.

viii. Toxic Proteins

a. These proteins hydrolyze or degrade enzymes.
b. **Example:** snake venom, ricin.

2.4.9 Milk Proteins:

a. Milk Protein contains about 0.6–0.7% protein which is not precipitated on acidification to pH 4.7.
b. This represents about 20% of the protein contained in skim milk. These whey proteins are separated into 2 fractions: lactalbumin and lactoglobulin.
c. The name casein is assigned to the fraction precipitated by acidifying milk to a pH of 4.7. It is present in cow's milk (3-3.5%) and human milk (0.3-0.6%).
d. Casein may be further purified by redissolving and precipitating again. It is of 3 types: α, β and γ.

2.4.10 Function of Proteins:

a. Proteins are seen in muscles, hair, skin and other tissues; they constitute the bulk of body's non-skeletal structure. Example: The protein keratin is present in nails and hair.
b. Some proteins are hormones and regulate many body functions. Example: Insulin hormone is a protein and it regulated the blood sugar level.
c. Some proteins act enzymes, they catalyze or help in biochemical reactions. Example: Pepsin and Tripsin.
d. Some proteins act as antibodies; they protect the body from the effect of invading species or substances.
e. Proteins transport different substances in blood of different tissues. Example: Haemoglobin is an oxygen transport protein.
f. Contractile proteins help in contraction of muscle and cells of our body. Example: Myosin is contractile protein.

Fibrinogen a glycoprotein helps in healing of wounds. It prevents blood loss and inhibits passage of germs.

FUNCTION OF PROTEINS

Class of Protein	Function in the Body	Examples
Structural	Provide structural components	*Collagen* is in tendons and cartilage. *Keratin* is in hair, skin, wool, and nails.
Contractile	Movement of muscles	*Myosin* and *actin* contract muscle fibers.
Transport	Carry essential substances throughout the body	*Hemoglobin* transports oxygen. *Lipoproteins* transport lipids.
Storage	Store nutrients	*Casein* stores protein in milk. *Ferritin* stores iron in the spleen and liver.
Hormone	Regulate body metabolism and nervous system	*Insulin* regulates blood glucose level. *Growth hormone* regulates body growth.
Enzyme	Catalyze biochemical reactions in the cells	*Sucrase* catalyzes the hydrolysis of sucrose. *Trypsin* catalyzes the hydrolysis of proteins.
Protection	Recognize and destroy foreign substances	*Immunoglobulins* stimulate immune responses.

2.5 Chapter at a Glance:

Term	Description
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
LDL	Low Density Lipoproteins
HDL	High density Lipoproteins
VLDL	Very low density lipoproteins
CHD	Coronary Heart Disease

2.6 Exercises:

2.6.1 Multiple Choice Questions:

1. Which biomolecule is distributed more widely in a cell?

   (a) Chloroplast (b) RNA
   (c) DNA (d) Spaherosomes

2. Which is a reducing sugar?

   (a) Galactose (b) Gluconic acid
   (c) Sucrose (d) -methyl galactosidase

3. Most abundant RNA in the cell

   (a) r-RNA (b) m-RNA
   (c) t-RNA (d) t-RNA threonine

4. Name the simplest amino acid

   (a) Alanine (b) Tyrosine
   (c) Asparagine (d) Glycine

5. Mineral associated with cytochrome is

   (a) Mg (b) Cu and Ag
   (c) Fe (d) Cu

6. the most common secondary structure of proteins is

   (a) B -pleated sheet (b) β-pleated sheet parallel
   (c) β -pleated sheet non-parallel (d) α-helix

7. The term enzyme was coined by

   (a) Urey Miller (b) Pasteur
   (c) Kuhne (d) Buchner

8. β-oxidation occurs in

   (a) Nucleus (b) Cytoplasm
   (c) Mitochondria (d) Chloroplast

9. Koshland's theory of enzyme action is known as

   (a) Lock and key theory (b) Reduced fit theory
   (c) Induced fit theory (d) Enzyme coenzyme theory

10. High content of triglycerides are found in

(a) VLDL
(b) LDL
(c) HDL
(d) Chylomicrons

2.6.2 Short Answer Questions:

1. Y. Electron transport chain (ETC) and its mechanism.
2. Y. Give the account of Oxidative phosphorylation & its mechanism and substrate phosphorylation.
3. Y. Give the account of Inhibitors ETC
4. Y. Write a note on oxidative decouplers.
5. Y. Write a note on Gluconeogenesis- Pathway and its significance.

2.6.3 Long Answer Questions:

1. Z. Define Glycolysis – Pathway, energetics and significance.
2. Z. Define Citric acid cycle- Pathway, energetics and significance.
3. Z. Give the detailed account HMP shunt and its significance.
4. Z Give the detailed importance of Glucose-6-Phosphate dehydrogenase (G6PD) and its deficiency.
5. Z. Give the detailed account of Hormonal regulation of blood glucose level and Diabetes mellitus.

Answer key MCQs

(1) – (b), (2) – (a), (3) – (a), (4) – (d), (5) – (c), (6) – (d), (7) – (c), (8) – (c), (9) – (c), (10) – (d).

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Chapter 3

Carbohydrate Metabolism

3.1 Introduction:

Glucose is the major form of sugar moiety present in blood and other body fluids. The digestion of food carbohydrates, such as starch, sucrose, and lactose produces the monosaccharides glucose, fructose and galactose, which pass into the blood stream. The study of synthesis (Anabolism) and degradation (Catabolism) of biomolecules is biochemically termed as metabolism.

Anabolism + Catabolism = *Metabolism*

(Synthesis) (Degradation)

Since glucose is the most important carbohydrate existing in physiological amounts in the body and is easily absorbed from the diet, the metabolism of carbohydrate resolves itself to the study of the metabolism of glucose and its main derivatives. The monosaccharides galactose and fructose are converted to glucose in the liver. All the monosaccharides are completely absorbed in the small intestine.

The glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body and used for the production of energy. Normally carbohydrate metabolism supplies more than half of the energy requirements of the body. In fact the brain largely depends upon carbohydrate metabolism as a source of energy and quickly ceases to function properly when the blood glucose level falls much below normal.

The major function of carbohydrate in metabolism is to serve as fuel and get oxidised to provide energy for other metabolic processes. The metabolic intermediates are used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

a) Glycolysis
b) Tricarboxylic acid cycle.

3.2 Glycolysis:

- Oxidation of glucose to pyruvate is called ***glycolysis***. It was first described by ***Embden-Meyerho***f and ***Parnas***. Hence it is also called as ***Embden-Meyerh*** of pathway. Glycolysis occurs virtually in all tissues. Erythrocytes and nervous tissues derive the energy mainly from glycolysis.

This pathway is unique in the sense that it can proceed in both aerobic (presence of O_2) and anaerobic (absence of O_2) conditions. All the enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.

- The overall equation for glycolysis from glucose to lactate is as follows:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{L}(+) - \text{Lactate} + 2\text{ATP} + 2\text{H}_2\text{O}$$

3.2.1 Reactions of Glycolytic Pathway:

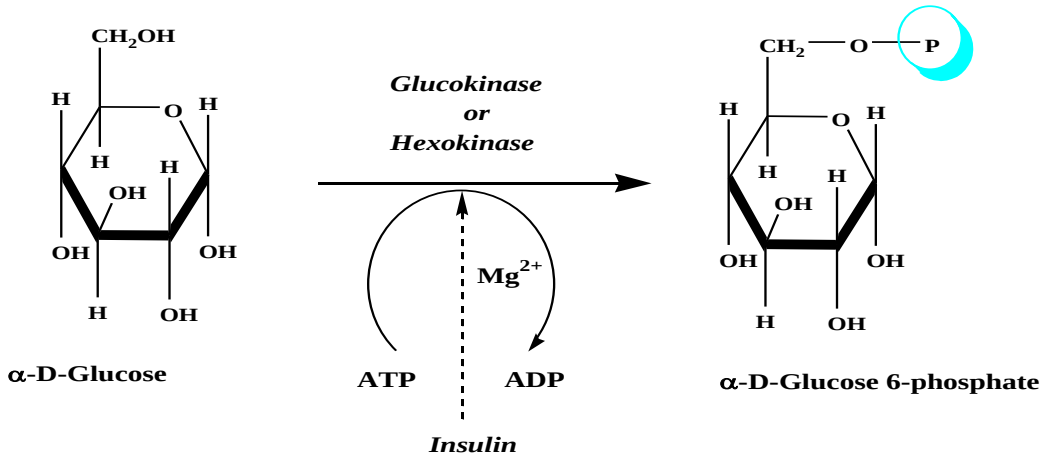
Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

Stage I: This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1, 6-diphosphate by donation of two phosphate groups from ATP.

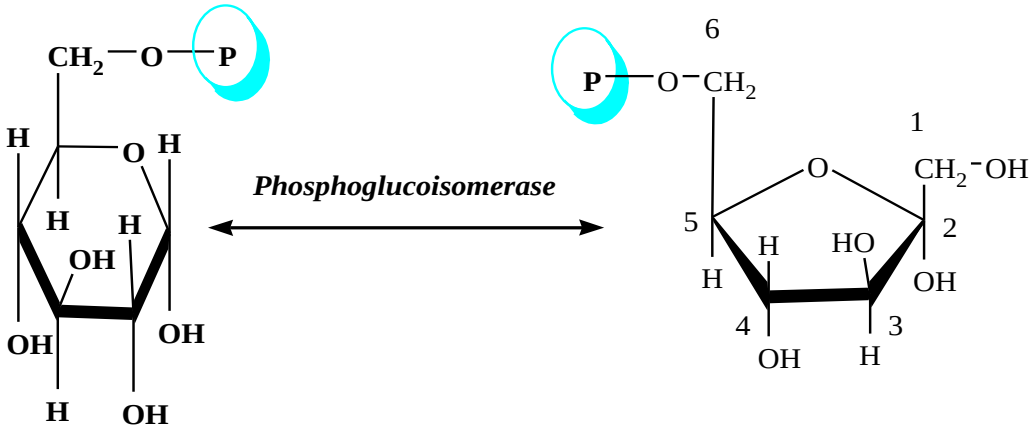
Uptake of glucose by cells and its phosphorylation:

Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport.

In other tissues ***insulin*** facilitates the uptake of glucose. Glucose is phosphorylated to form **glucose 6-phosphate**. The enzyme involved in this reaction is *glucokinase or hexokinase*. This reaction is irreversible.



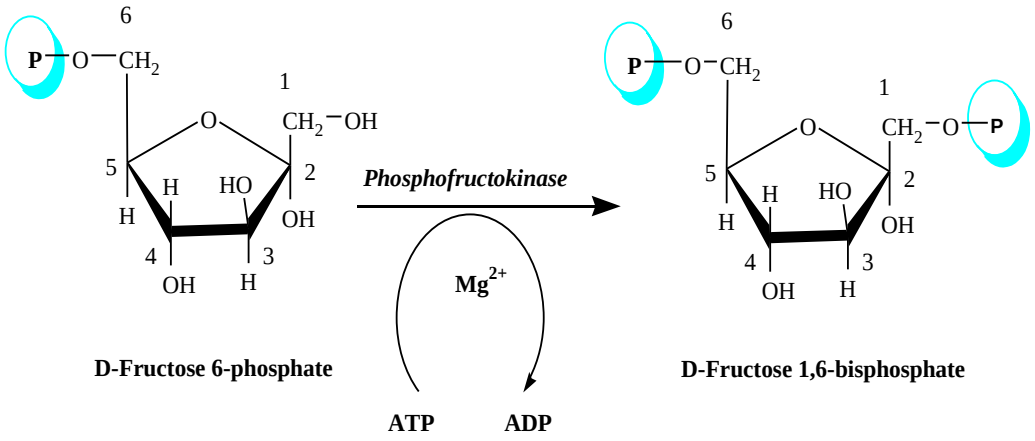
Conversion of glucose 6-phosphate to fructose 6-phosphate: Glucose 6-phosphate is converted to fructose 6-phosphate by the enzyme phosphoglucoisomerase.



α-D-Glucose 6-phosphate **D-Fructose 6-phosphate**

Conversion of fructose 6-phosphate to fructose 1, 6 diphosphate:

Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme *phosphofructokinase* to produce fructose1, 6-diphosphate.

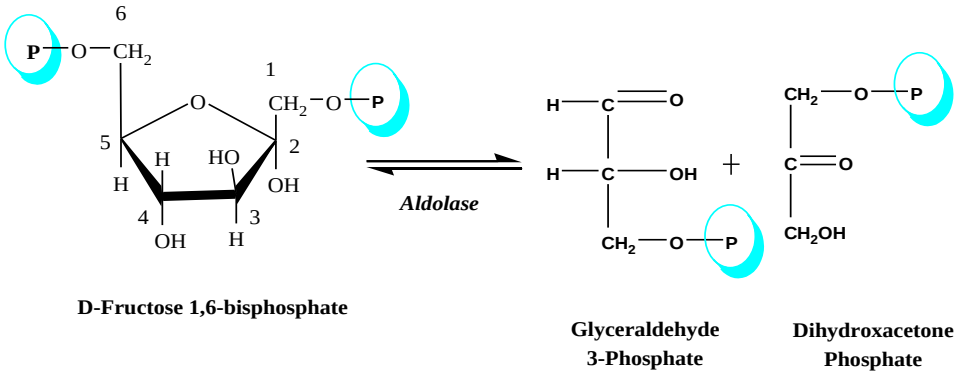


D-Fructose 6-phosphate **D-Fructose 1,6-bisphosphate**

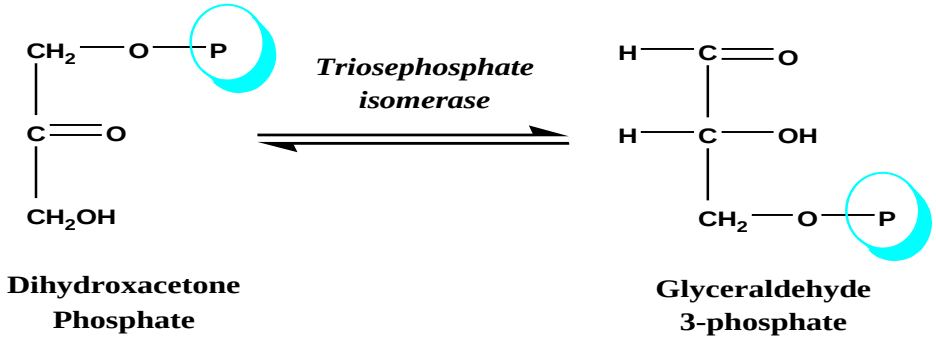
Stage II: Actual splitting of fructose 1, 6 diphosphate:

Fructose 1, 6 diphosphate is split by the enzyme *aldolase* into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one *ketotriose* - dihydroxy acetone phosphate.

The reaction is reversible. There is neither expenditure of energy nor formation of ATP.



Interconversion of triose phosphates: Both triose phosphates are interconvertible.

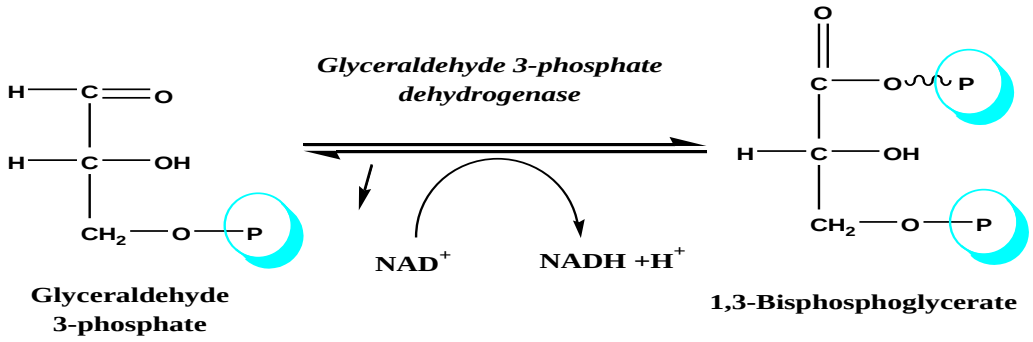


Stage III: It is the energy yielding stage. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

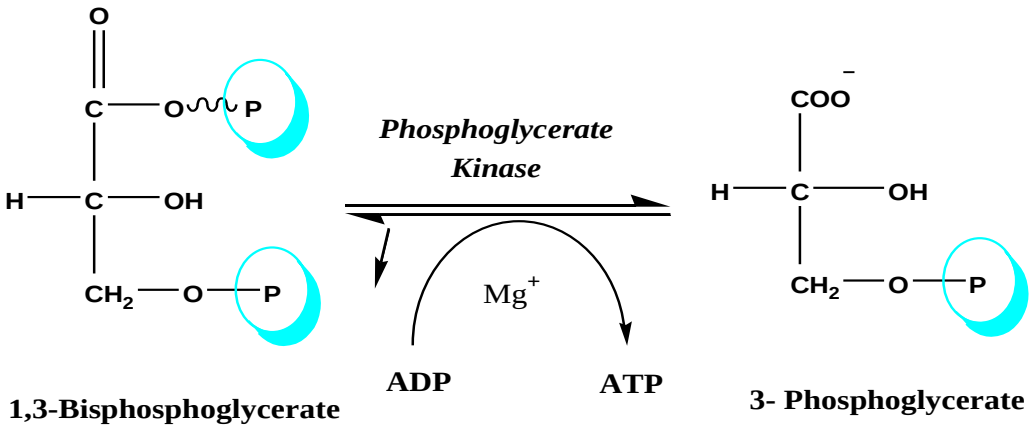
Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate:

Glycolysis proceeds by the oxidation of glyceraldehyde 3-phosphate to form 1, 3−bisphosphoglycerate.

The reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphate dehydrogenas*.



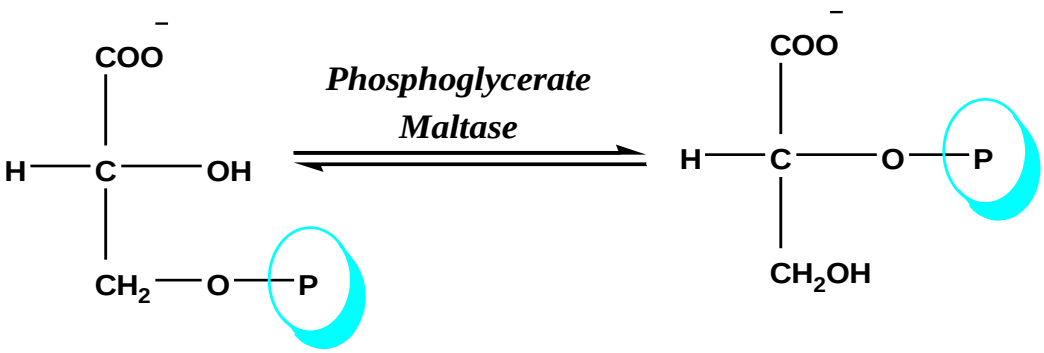
Conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate: The reaction is catalyzed by the enzyme *phosphoglycerate kinase*. The high energy phosphate bond at position-1 is transferred to ADP to form ATP molecule.



Stage IV: It is the recovery of the phosphate group from *3-phosphoglycerate*. The two molecules of *3- phosphoglycerate*, the end product of the previous stage, still retains the phosphate group, originally derived from ATP in Stage I.

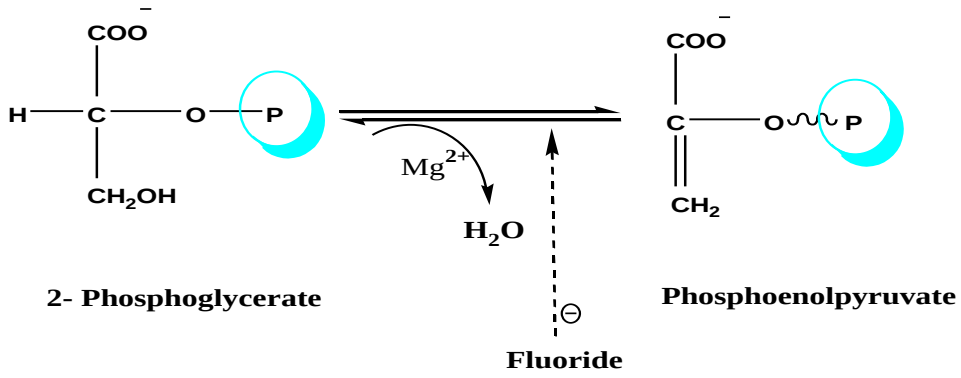
Conversion of 3-phosphoglycerate to 2-phosphoglycerate:

3-phosphoglycerate formed by the above reaction is converted to 2-phosphoglycerate, catalyzed by the enzyme *phosphoglycerate mutase*.

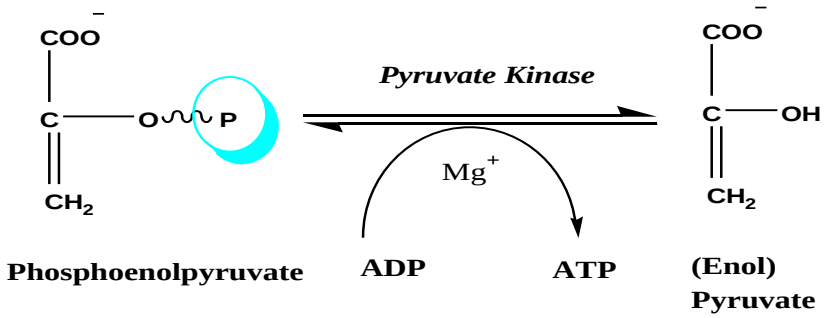


Conversion of 2-phosphoglycerate to phosphoenol pyruvate:

The reaction is catalyzed by the enzyme *enolase*, the enzyme requires the presence of either Mg^{2+} or Mn^{2+} ions for activity.



Conversion of phosphoenol pyruvate to pyruvate: *Phosphoenol pyruvate* is converted to *pyruvate*, the reaction is catalysed by the enzyme *pyruvate kinase*. The high energy phosphate group of phosphoenol pyruvate is directly transferred to ADP, producing ATP. The reaction is irreversible.



3.2.2 Summary of Glycolysis:

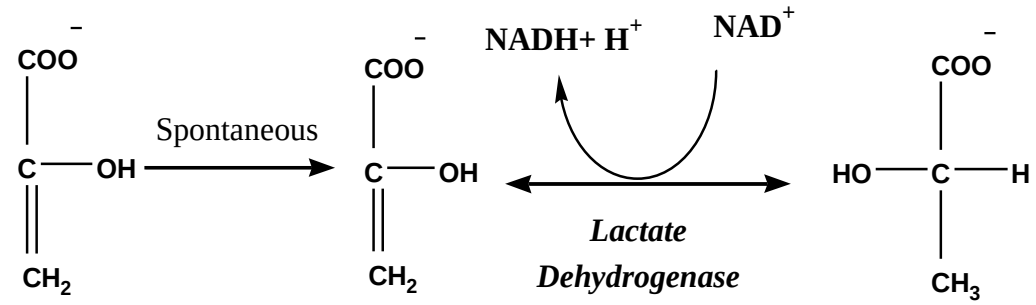
During glycolysis NAD^+ is reduced to NADH. At the same time, *glyceraldehyde 3-phosphate* is oxidized to *1, 3-bisphosphoglycerate*. To conserve the coenzyme NAD^+, NADH must be reoxidized.

Under anaerobic conditions this is done when pyruvic acid is converted to lactic acid. In the presence of oxygen, NADH, can be oxidized to NAD^+ with the help of the respiratory enzymes.

3.3 Anaerobic Phase:

In the absence of O_2, reoxidation of NADH at *glyceraldehyde 3-phosphate dehydrogenase* stage cannot take place in respiratory chain. But the cells have limited coenzyme.

Hence to continue the glycolysis **NADH** must be reoxidized to **NAD^+**. This is achieved by reoxidation of NADH by conversion of pyruvate to lactate (without producing ATP).



(Enol) Pyruvate **(Keto) Pyruvate** **L(+)-Lactate**

It is to be noted that in the reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*, therefore, no ATP is produced.

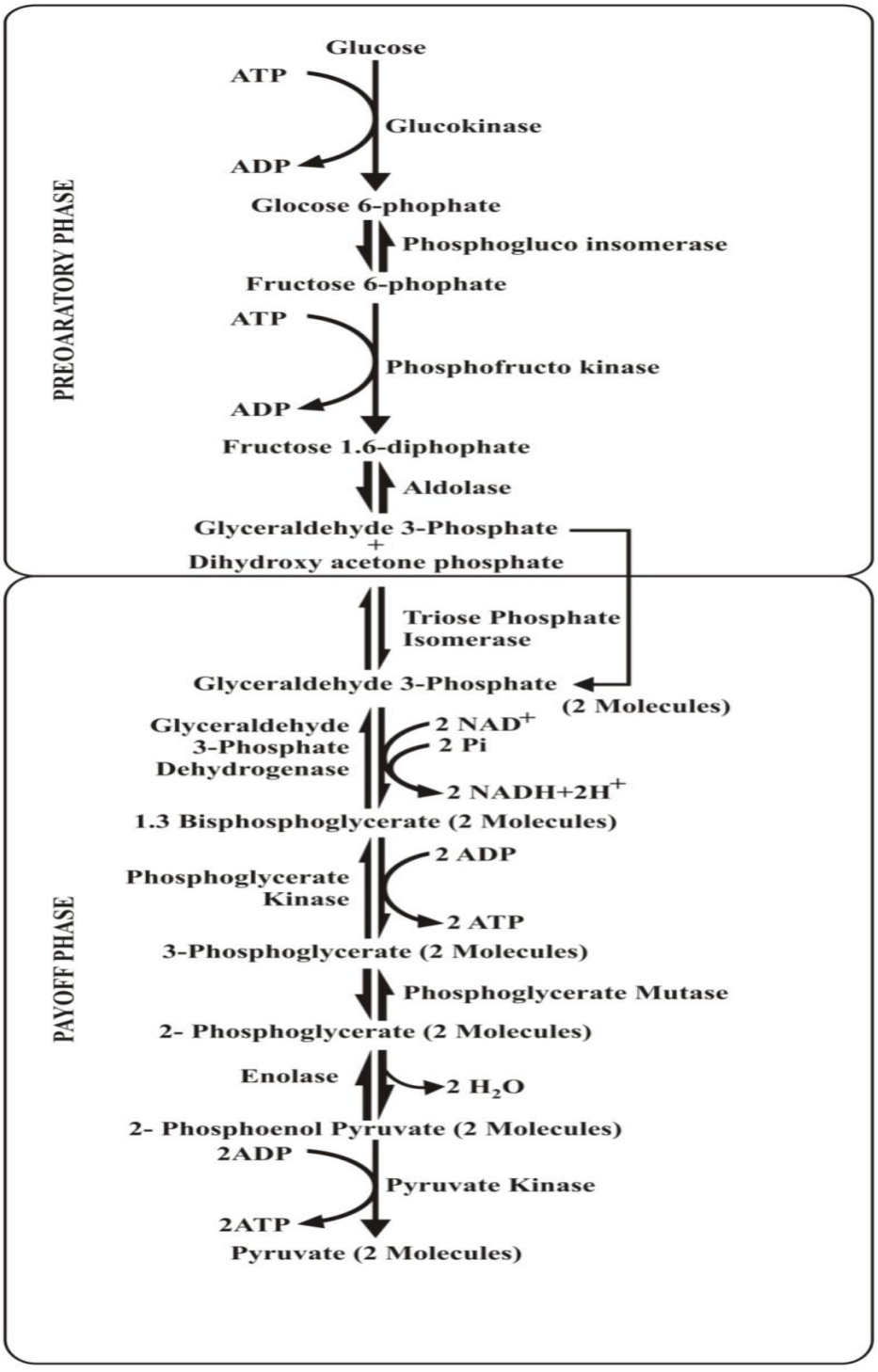
In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

3.4 Energy Yield per Glucose Molecule Oxidation:

During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

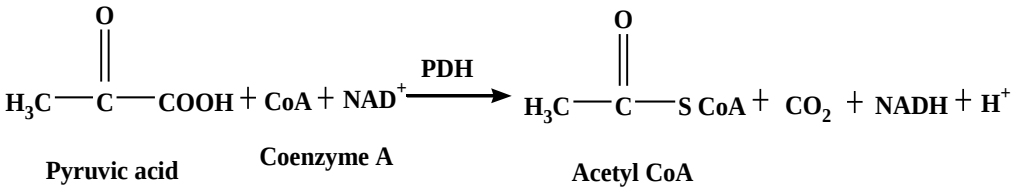
Reactions Catalyzed	***ATP used***	***ATP formed***
Stage I: 1. Glucokinase (for phosphorylation)	1	
2. Phosphofructokinase I (for phosphorylation)	1	
Stage II: 3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain)		6
4. Phosphoglycerate kinase (substrate level phosphorylation)		2
Stage IV: 5. Pyruvate kinase (substrate level phosphorylation)		2
Total	**2**	**10**
Net gain		**08**

A. Schematic Diagram of Glycolytic Pathway:



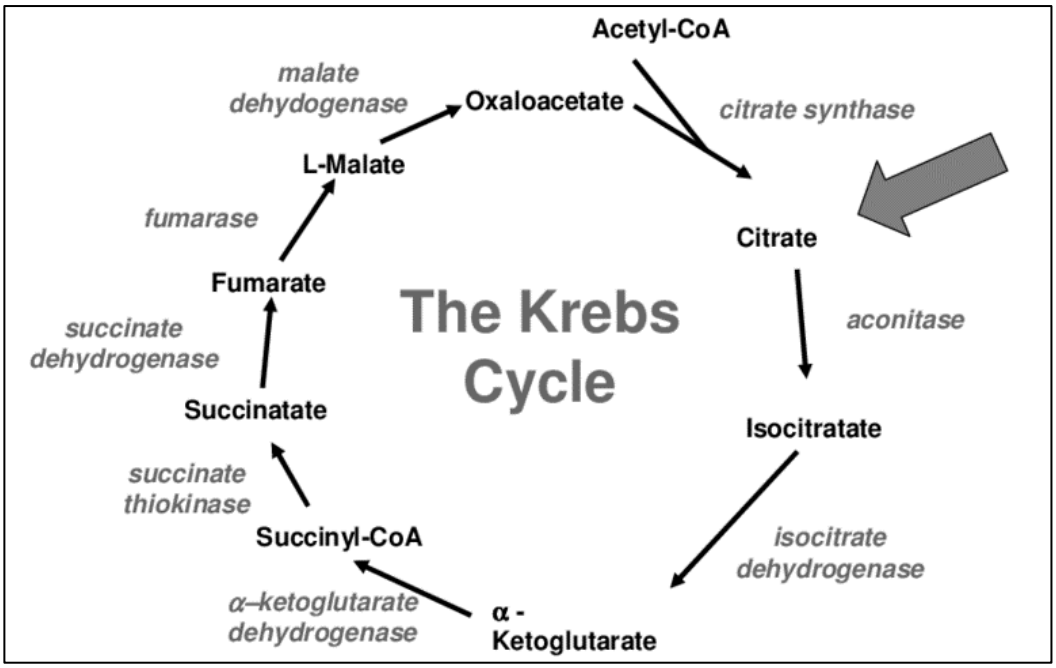
B. Tricarboxylic Acid Cycle (TCA Cycle):

This cycle is the aerobic phase of carbohydrate metabolism and follows the anaerobic pathway from the stage of pyruvate and is called as citric acid cycle or TCA cycle. The name citric acid cycle stems from citric acid which is formed in the first step of this cycle. This cycle is also named "Kerbs cycle" after H.A. Krebs, an English biochemist who worked on it. Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl coenzyme A (active acetate) before entering the citric acid cycle. This occurs in the mitochondrial matrix and forms a link between glycolysis and TCA cycle. This reaction is catalysed by the multienzyme complex known as pyruvate dehydrogenase complex.



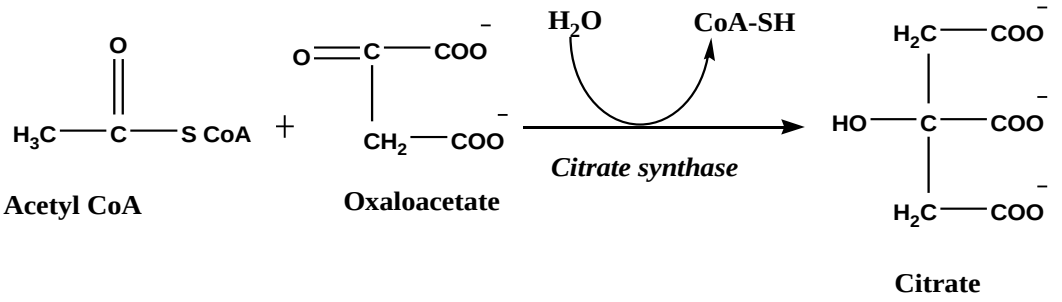
PDH- Pyruvate dehydogenase

C. Schematic Diagram of Krebs Cycle:

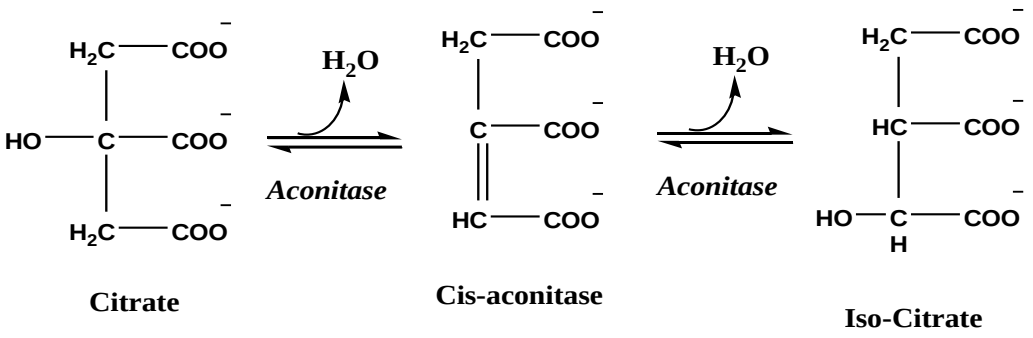


i. Reactions of the Citric Acid Cycle: There are eight steps in the cycle and the reactions are as follows.

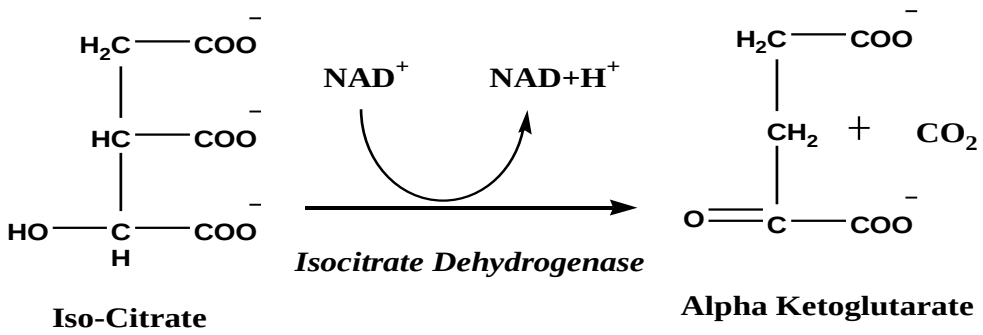
ii. Formation of Citrate: The first reaction of the cycle is the condensation of acetyl CoA with oxaloacetate to form citrate, catalyzed by *citrate synthase*. This is an irreversible reaction.



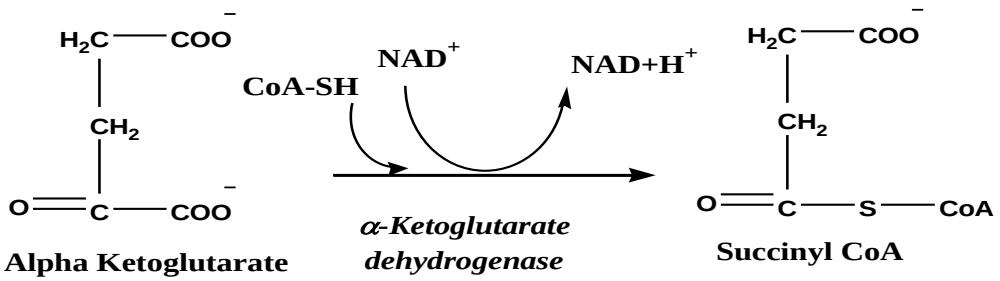
iii. Formation of Isocitrate via Cis Aconitate*:* The enzyme *aconitase* catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis-aconitate.



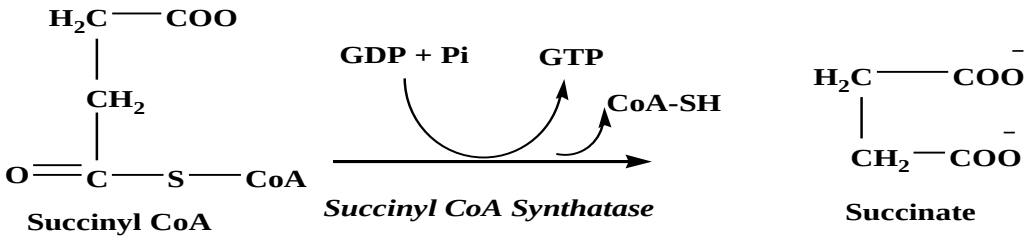
iv. Oxidation of Isocitrate to α-Ketoglutarate and CO_2: In the next step, *isocitrate dehydrogenase* catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.



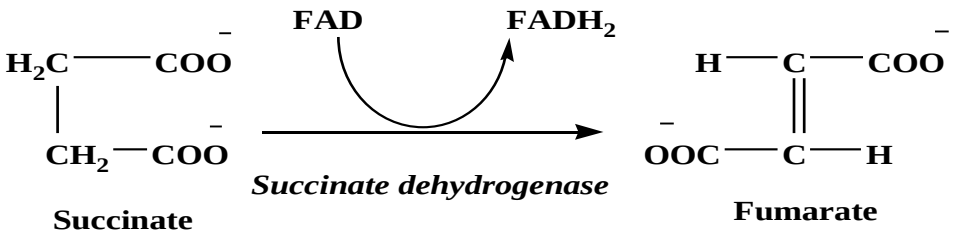
v. Oxidation of α-keto Glutarate to Succinyl CoA and CO_2: The next step is another oxidative decarboxylation, in which *α*-ketoglutarate is converted to succinyl CoA and CO_2 by the action of the *α-ketoglutarate dehydrogenase* complex. The reaction is irreversible.



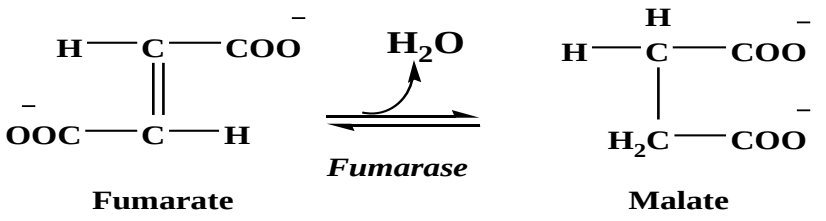
vi. Conversion of Succinyl CoA to Succinate: The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation) and the enzyme that catalyzes this reversible reaction is called *succinyl CoA synthetase* or *succinic thiokinase*.



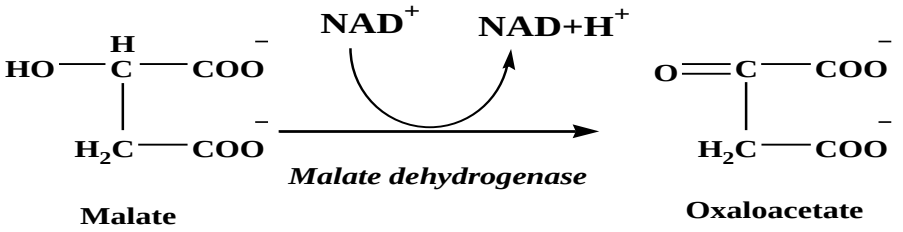
vii. Oxidation of Succinate to Fumarate: The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme *succinate dehydrogenase*.



viii. Hydration of Fumarate to Malate: The reversible hydration of fumarate to malate is catalyzed by *fumarase*.



ix. Oxidation of Malate to Oxaloacetate: The last reaction of the citric acid cycle is, NAD linked *malate-dehydrogenase* which catalyses the oxidation of malate to oxaloacetate.



x. Energy Yield from TCA Cycle: If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through $FADH_2$, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate ($2\ NADH + 2H^+$) (2×3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA ($2\ NADH + 2H^+$) (2×3)	6
3. 2 succinyl CoA → 2 succinate ($2\ GTP = 2ATP$)	2
4. 2 succinate → 2 Fumarate ($2\ FADH_2$) (2×2)	4
5. 2 malate → 2 oxaloacetate ($2\ NADH + 2H^+$) (2×3)	6
Total No.of ATP formed	24

3.5 Vitamins Play Key Roles in the Citric Acid Cycle:

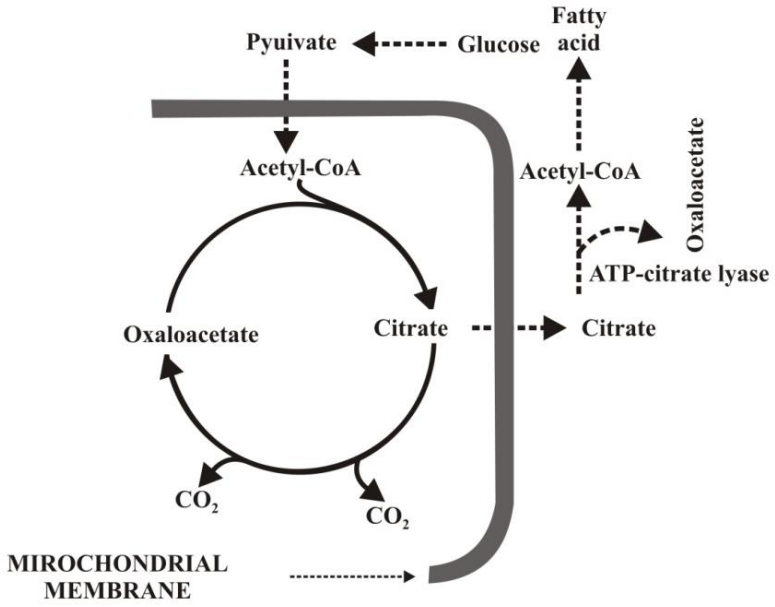
Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism:

a. **Riboflavin**, in the form of flavin adenine dinucleotide (FAD), a cofactor in the-ketoglutarate dehydrogenase complex and in succinate dehydrogenase.
b. **Niacin**, in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for three dehydrogenases in the cycle — isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase.
c. **Thiamin (vitamin B1),** as thiamin diphosphate, the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.
d. **Pantothenic Acid,** as part of coenzyme A, the cofactor attached to ―activ‖ carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

e. **The Citric Acid Cycle Takes Part in Fatty Acid Synthesis:** Acetyl-CoA, formed from pyruvate by the action of *pyruvate dehydrogenase*, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.)

Pyruvate dehydrogenase is a mitochondrial enzyme and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl-CoA.

Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by *ATP-citrate lyase*.



Participation of the citric acid cycle in fatty acid synthesis from glucose

3.6 HMP Shunt ATHWAY:

Although glycolysis and citric acid cycle are the common pathways by which animal tissues oxidise glucose to CO_2 and H_2O with the liberation of energy in the form of ATP, a number of alternative pathways are also discovered.

The most important one is Hexose Monophosphate Shunt Pathway (HMP shunt). The p a t h w a y occurs in the extra mitochondrial soluble portion of the cells.

It has two major functions:

The formation of NADPH for synthesis of fatty acids and steroids

The synthesis of ribose for nucleotide and nucleic acid formation.

The fundamental difference between NADPH and NADH (reduced nicotinamide adenine dinucleotide) is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis, for example in the biosynthesis of fatty acids and steroids.

Glucose, fructose and galactose are the main hexoses absorbed from the gastrointestinal tract, derived principally from dietary starch, sucrose and lactose respectively. Fructose and galactose are converted to glucose, mainly in the liver.

The overall equation of the hexose phosphate pathway is

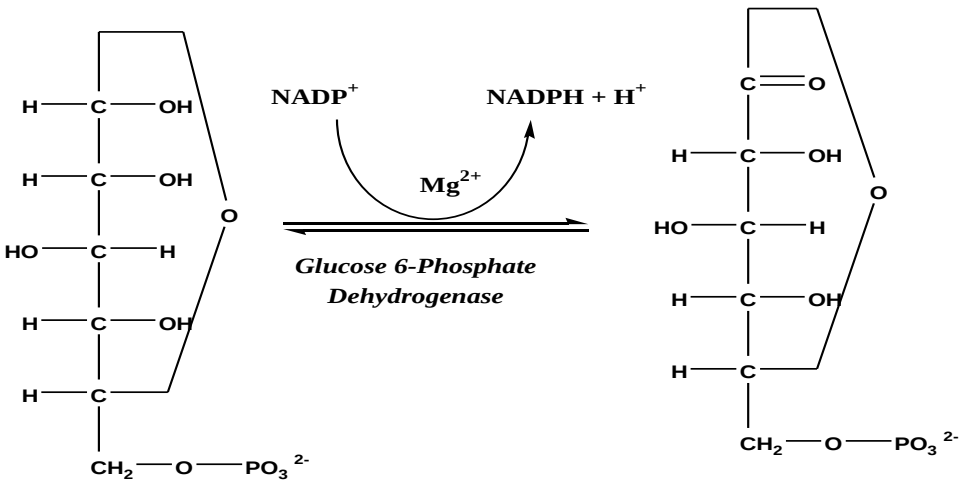
$$\text{Glucose 6-phosphate} + 2NADP^{+} + H_2O \longrightarrow \text{Ribose 5-phosphate} + CO_2 + 2NADPH + 2H^{+}$$

And the net result is the production of NADPH, a reductant for biosynthetic reactions and ribose 5- phosphate, a precursor for nucleotide synthesis.

3.7 Oxidative Reactions of the Hexose Mono-Phosphate Pathway:

Step 1:

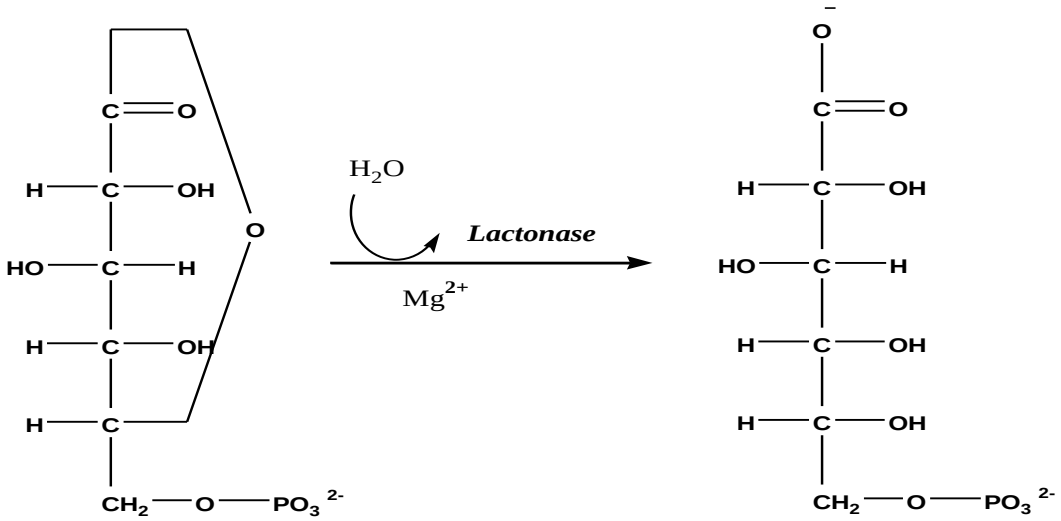
Glucose 6-phosphate in the presence of $NADP^{+}$ and the enzyme *glucose 6-phosphate dehydrogenase*, forms 6-phospho glucono δ-lactone. The first molecule of $NADPH^{+}$ is produced in this step.



Glucose 6-Phosphate **6-Phosphate gluco delta lactone**

Step 2:

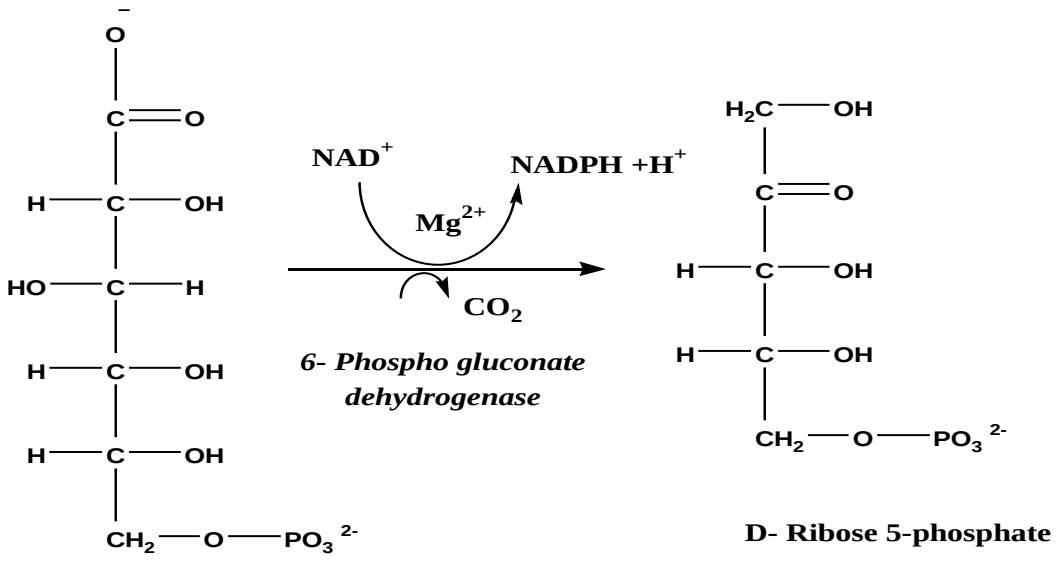
The 6-phospho glucono- δ-lactone is unstable and the ester spontaneously hydrolyses to 6- phosphogluconate. The enzyme that catalyses the reaction is lactonase.



6-Phosphate gluco delta lactone **6-phospho gluconate**

Step 3:

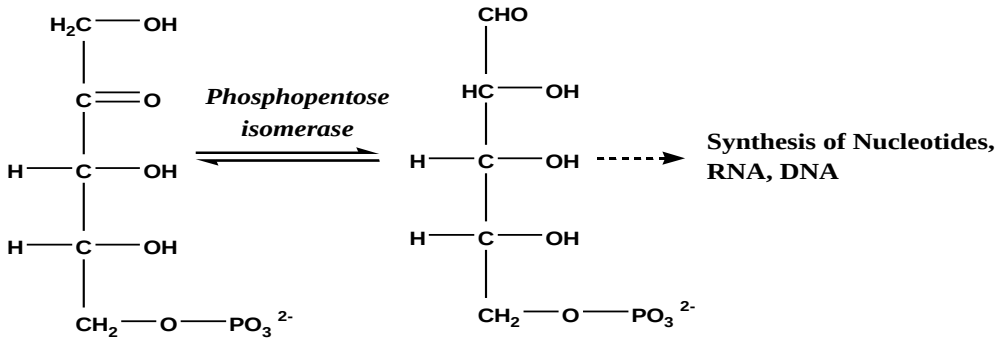
6-phospho gluconate further undergoes dehydrogenation and decarboxylation by 6-phosphogluconate dehydrogenase to form the ketopentose, D-ribulose 5-phosphate. This reaction generates the second molecule of NADPH.



6-phospho gluconate **D- Ribose 5-phosphate**

Step 4:

The enzyme phosphopentose isomerase converts ribulose 5-phosphate to its aldose isomer, D-ribose 5-phosphate.



Ribulose 5-phosphate **D-Ribose 5-Phosphate**

Note By

Genetic deficiency of *glucose 6-phosphate dehydrogenase*, the first enzyme of the pentose phosphate pathway, is a major cause of hemolysis of red blood cells, resulting in hemolytic anemia and Glucuronic acid is synthesized from glucose via the uronic acid pathway, of major significance for the excretion of metabolites and foreign chemicals (xenobiotics) as glucuronides.

3.8 Gluconeogenesis:

The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis. The major site of gluconeogenesis is liver. It usually occurs when the carbohydrate in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet and at the time of starvation, when tissue proteins are broken down to amino acids.

3.8.1 Substrates for Gluconeogenesis:

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include the intermediates of glycolysis and the citric acid cycle. Glycerol, lactate, and the α-keto acids obtained from the deamination of glucogenic amino acids are the most important gluconeogenic precursors.

a. Glycerol: Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue and in the liver. Glycerol is phosphorylated by glycerol kinase to glycerol phosphate, which is oxidized by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate as an intermediate of glycolysis.

b. Lactate: It is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells. In the Cori cycle, glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which diffuse into the circulation.

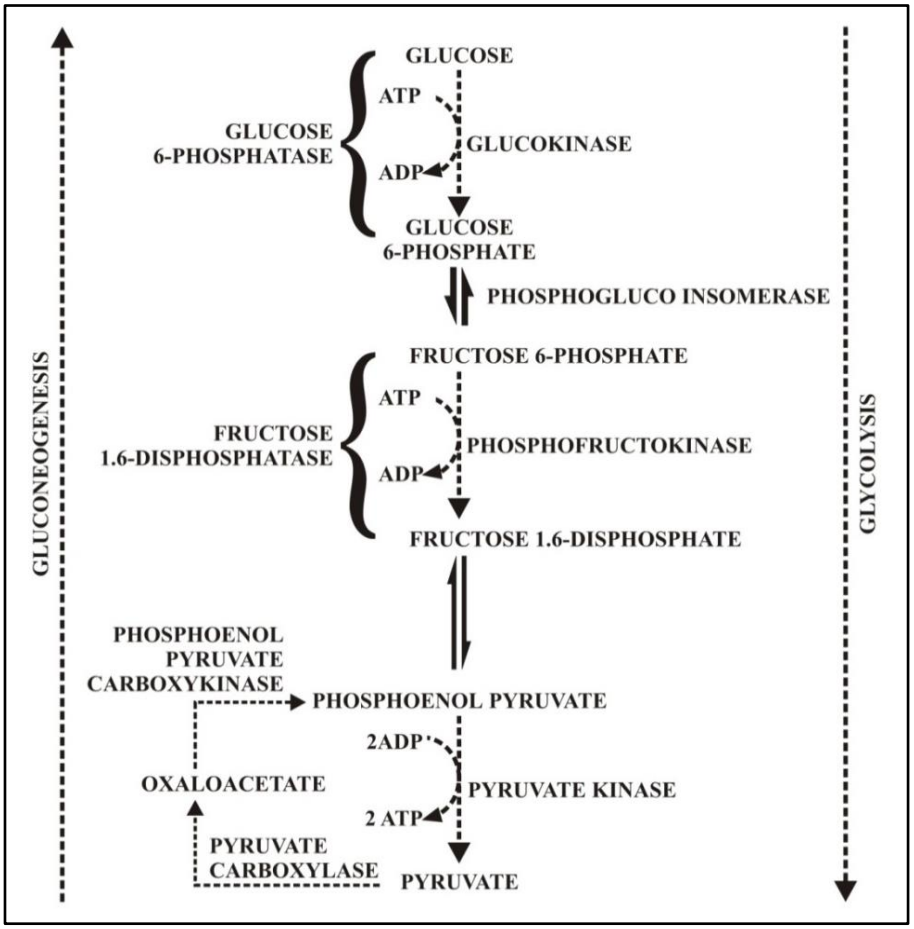
c. Amino Acids: Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during fasting.

3.8.2 Gluconeogenesis and Glycolysis:

- Gluconeogenesis and glycolysis are opposing metabolic pathways and share a number of enzymes. In glycolysis, *glucose* is converted to *pyruvate* and in gluconeogenesis *pyruvate* is converted to *glucose*. However gluconeogenesis is not exact reversal of glycolysis.
- There are three essentially irreversible steps in glycolysis which are

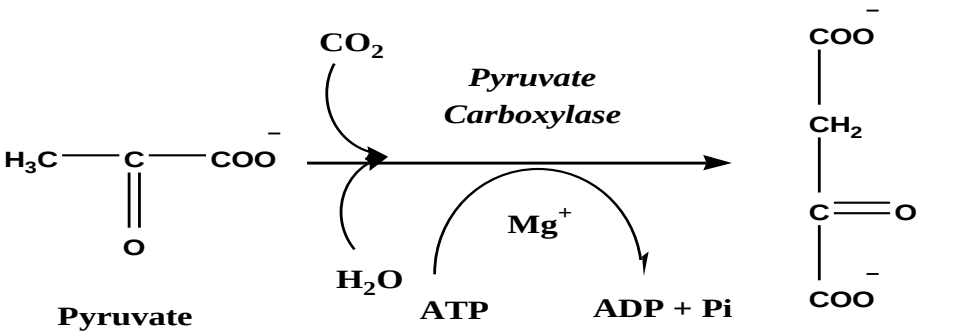
1. Glucose + ATP —(Glucokinase)→ Glucose 6-phosphate + ADP
2. Fructose 6-phosphate + ATP —(Phosphofructo kinase)→ Fructose 1,6-diphosphate + ADP
3. Phosphoenol pyruvate + ADP —(Pyruvate kinase)→ Pyruvate + ATP

- In gluconeogenesis these three reactions are bypassed or substituted by the following news ones.



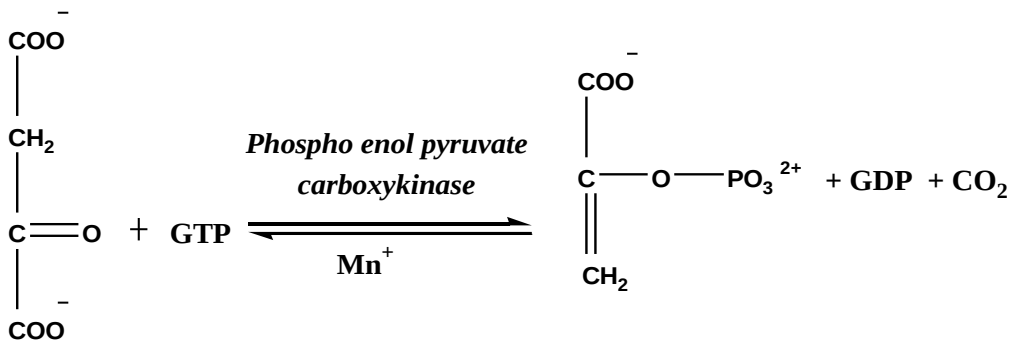
- **Reactions of gluconeogenesis:**

1. The formation of phosphoenol pyruvate begins with the carboxylation of pyruvate at the expense of ATP to form Oxalo acetate.



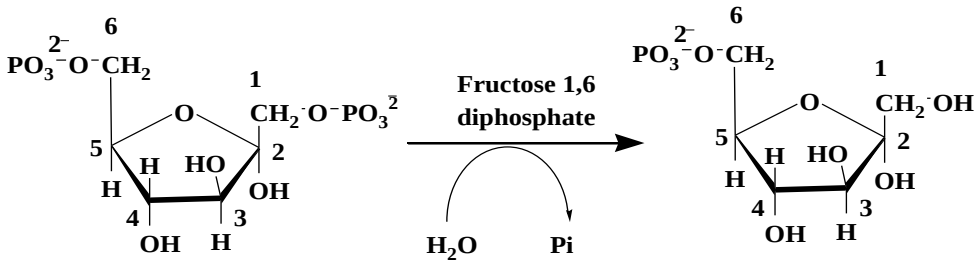
Pyruvate **Oxalo acetate**

Oxalo acetate is converted to phosphoenolpyruvate by phosphorylation with GTP, accompanied by a simultaneous decarboxylation.



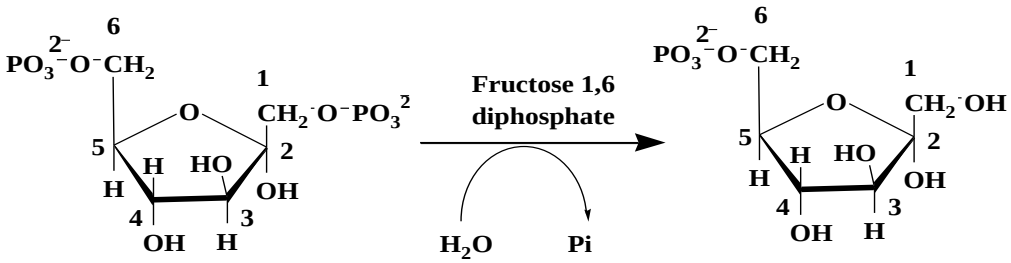
Oxalo acetate **Phosphoenol pyruvate**

Fructose 6-phosphate is formed from fructose 1, 6-diphosphate by hydrolysis and the enzyme fructose 1, 6 diphosphatase catalyses this reaction.



D-Fructose 1,6 diphosphate **D-Fructose 6-biphosphate**

Glucose is formed by hydrolysis of glucose 6-phosphate catalysed by glucose 6-phosphatase.



D-Fructose 1,6 diphosphate **D-Fructose 6-biphosphate**

3.8.3 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.

3.8.4 Gluconeogenesis of Propionate:

Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl CoA.

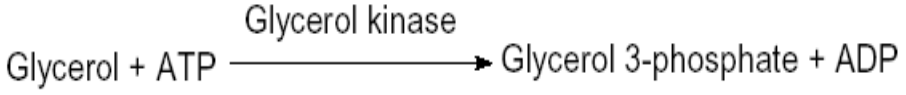
3.8.5 Gluconeogenesis of Glycerol:

At the time of starvation glycerol can also undergo gluconeogenesis.

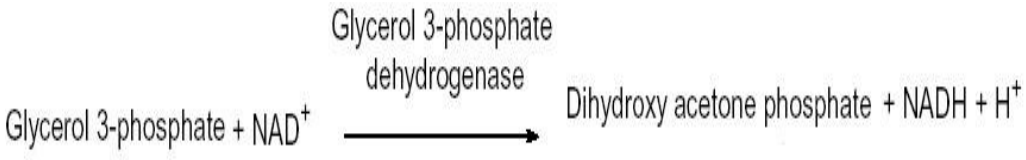
When the triglycerides are hydrolysed in the adipose tissue, glycerol is released.

Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it.

Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.



This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD^+ and glycerol 3-phosphate dehydrogenase.

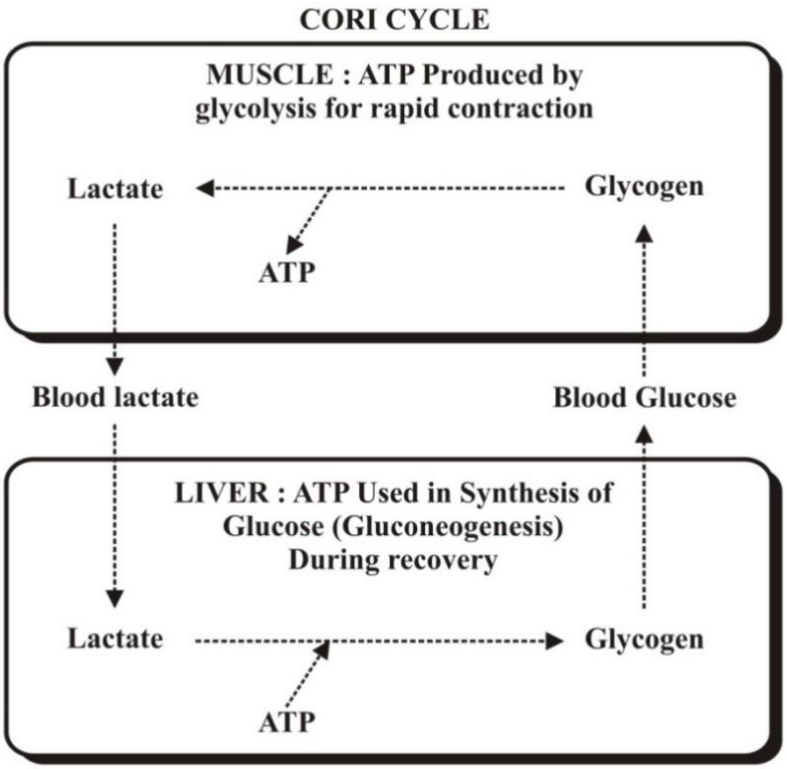


This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose.

Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

3.8.6 Gluconeogenesis of Lactic Acid (Cori cycle):

The liver and skeletal muscles exhibit a special metabolic cooperation as far as carbohydrates are concerned by the way of a cycle of conversions known as Cori cycle.



In this cycle liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactate produced by the active skeletal muscles.

At the time of heavy muscular work or strenuous exercise, O_2 supply is inadequate in active muscles but the muscles keep contracting to the maximum.

Hence, glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus lactate gets accumulated in the muscle.

Muscle tissue lacks the enzyme *glucose 6-phosphatase* hence it is incapable of synthesizing glucose from lactic acid and the conversion take place only in the liver.

Lactate diffuses out of the muscle and enters the liver through blood. In the liver lactate is oxidised to pyruvate which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose.

The glycogen may be once again converted to glucose (glycogenolysis) and may be recycled to the muscle through the blood. The process of gluconeogenesis completes the cycle by converting glucose once again to muscle glycogen.

3.9 Diabetes Mellitus:

Diabetes mellitus is an important disorder of carbohydrate metabolism. However, fat and protein metabolism are also affected in diabetic condition.

Diabetes means excretion of excessive volume of urine and mellitus means sweet. So the word diabetes mellitus refers to chronic excretion of large volume of urine containing glucose.

Diabetes mellitus, caused by a deficiency in the secretion or action of insulin, is a relatively common disease. Insulin is an endocrine hormone which is secreted by β-cells of *islets of Langerhans* of pancreas.

The abnormality in glucose metabolism is indicative of diabetes or a tendency towards the condition. Diabetes mellitus is really a group of diseases in which the regulatory activity of insulin is defective.

There are two major clinical classes of the disease:

Type - I or insulin dependent diabetes mellitus (IDDM), this disease begins early in the life and quickly becomes severe.

Type - II or non-insulin dependent diabetes mellitus (NIDDM), this disease is slow to develop, milder and often goes unrecognized.

Type one requires insulin therapy and careful, lifelong control of the balance between glucose intake and insulin dose. The decreased or defective production of insulin is characterized by the following symptoms.

Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.

A. Polyuria: This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).

B. Polydipsia: The excessive thirst which leads to increased consumption of water. This condition is known as polydipsia. This is to replace the volume of water excreted due to polyuria.

C. Polyphagia: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating, they lose weight and become lean and emaciated.

As glucose is not enough for energy production, increased mobilisation of fat from adipose tissue occurs.

But the metabolism of fat is incomplete resulting in the production of large amounts of the intermediary products of fat metabolism namely ketone bodies (e.g. Acetoacetate and β-hydroxybutarate). This condition is known as 'ketosis' and excess ketone bodies cause severe

Acidosis, ultimately resulting in 'coma'.

Deposition of lipids in the walls of the blood vessels resulting "atherosclerosis".

Biochemical measurements on the blood and urine are essential in the diagnosis and treatment of diabetes, which causes profound changes in metabolism. A sensitive diagnostic criterion is provided by the *glucose tolerance test* (GTT).

I. Classification of Antidiabetic Drugs: Antidiabetic drugs can be classified into two categories:

II. Insulin Injections: Mostly used on serious cases of diabetes.

III. Oral Hypoglycaemic Agents: These drugs are the agents which can be taken as single or in combination to lower the blood glucose in type 2 diabetes.

The type 2 diabetes mellitus is due to increased peripheral resistance to insulin or to reduced secretion of insulin. Oral hypoglycaemic along with good lifestyle changes may achieve good glycaemic control and it is customary to monitor such changes for three months before considering medication.

These agents are not prescribed generally in type 1 diabetes, but *metformin* may be of use in overweight type 1 diabetics.

The following groups of oral hypoglycaemics are currently available:

a. **Biguanides Derivatives:** Metformin;

b. **Sulphonylureas Derivatives:** Glimepiride, glipizide, glibenclamide;
c. **Postprandial Glucose Regulators:** Repaglinide and Nateglinide;
d. **Thiazolidinediones Derivatives:** Pioglitazone and Rosiglitazone and Acarbose: which acts by inhibiting intestinal alpha glucosidases which delays the absorption and digestion of sucrose and starch.

3.10 Glucose Tolerance Test (GTT) or Oral Glucose Tolerance Test (OGTT):

A Glucose Tolerance Test in medical practice is the administration of glucose to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia. The glucose is most often given orally so technically is terms as an oral glucose tolerance test (OGTT).

3.10.1 Preparation and Cautions:

The patient is instructed not to restrict carbohydrate intake in the days or weeks before the test. The test should not be done during an illness, as results may not reflect the patient's glucose metabolism when healthy. A full adult dose should not be given to a person weighing less than 43 kg (94 lb).

3.10.2 Procedure for OGTT:

The patient should have been fasting for the previous 8-14 hours (water is allowed).

Then the patient given a glucose solution to drink about 1.75 grams per kilogram of body weight, to a maximum dose of 75 g. It should be drunk within 5 minutes.

Blood is drawn at intervals for measurement of glucose (blood sugar), the intervals and number of samples varies according to the purpose of the test.

The urine samples may also be collected for testing along with the fasting and 2 hour blood tests if renal glycosuria (sugar excreted in the urine despite normal levels in the blood) is suspected,

3.10.3 Interpretation of OGTT Results:

- Fasting plasma glucose should be below 6.1 mmol/l (110 mg/dl). Fasting levels between 6.1 and 7.0 mmol/l (110 and 126 mg/dl) are borderline ("impaired fasting glycaemia"), and fasting levels repeatedly at or above 7.0 mmol/l (126 mg/dl) are diagnostic of diabetes.
- The 2 hour glucose level should be below 7.8 mmol/l (140 mg/dl). Levels between this and 11.1 mmol/l (200 mg/dl) indicate, "Impaired Glucose Tolerance." A glucose level above 11.1 mmol/l (200 mg/dl) at 2 hours confirms a diagnosis of diabetes.
- REGULATION OF GLUCONEOGENESIS

The regulation of gluconeogenesis is determined primarily by the circulating level of glucagon, and by the availability of gluconeogenic substrates.

3.11 Glucagon:

This pancreatic islet hormone stimulates gluconeogenesis by three mechanisms.

- **Changes in allosteric effectors:** Glucagon lowers the level of fructose 2, 6-bisphosphate, resulting in activation of *fructose 1, 6 - bisphosphatase* and inhibition of *phosphofructokinase,* thus favoring gluconeogenesis over glycolysis.
- **Covalent modification of enzyme activity:** Glucagon, via an elevation in cyclic AMP (cAMP) level and *cAMP- dependent protein kinase* activity, stimulates the conversion of *pyruvate kinase* to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose.
- **Induction of enzyme synthesis:** Glucagon increases the transcription of the *PEP- carboxykinase* gene, thereby increasing the availability of this enzyme's activity as levels of its substrate rise during fasting.

[***Note by:*** Insulin causes decreased transcription of the mRNA for this enzyme.]

A. Substrate Availability:

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis.

B. Allosteric activation by Acetyl CoA:

Allosteric activation of hepatic *pyruvate carboxylase* by acetyl CoA occurs during fasting. As a result of excessive lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and leads to activation of *pyruvate carboxylase*.

[**Note:** Acetyl CoA inhibits *pyruvate dehydrogenase*.Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle]

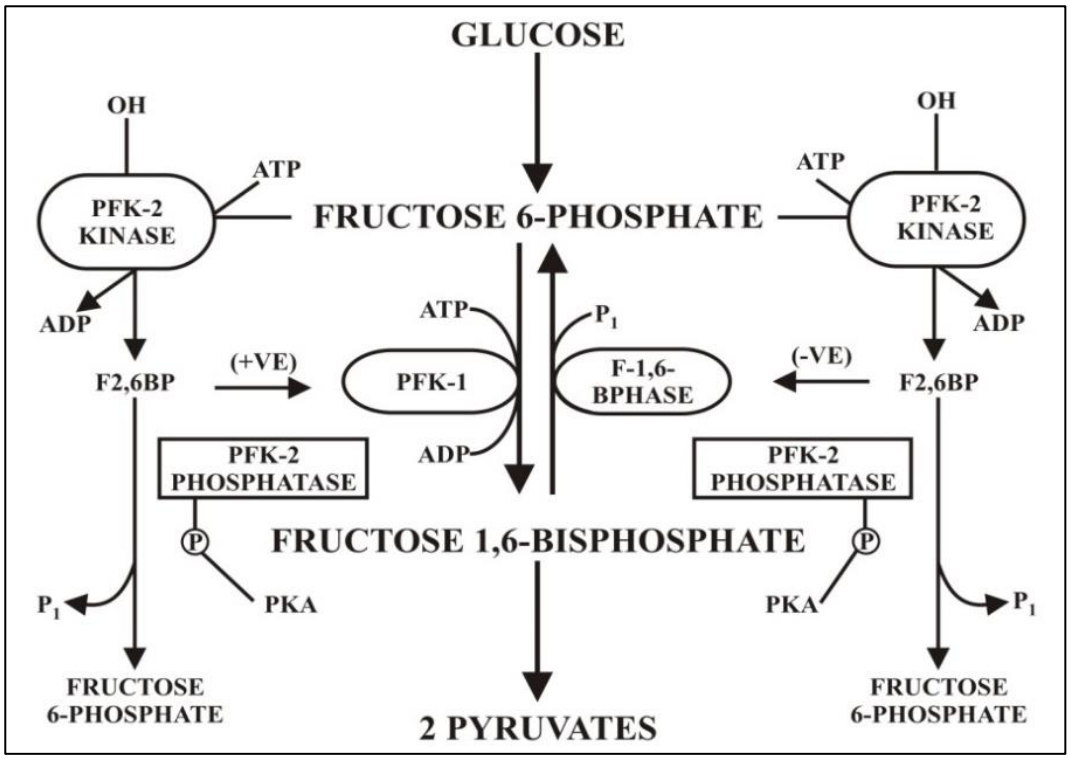
C. Allosteric inhibition by AMP:

Fructose 1, 6 - bisphosphatase is inhibited by AMP—a compound that activates phosphofructokinase. Elevated AMP thus stimulates pathways that oxidize nutrients to provide energy for the cell.

[**Note:** ATP and NADH, produced in large quantities during fasts by catabolic pathways, such as fatty acid oxidation, are required for gluconeogenesis. Fatty acid oxidation also provides the acetyl CoA that allosterically activates pyruvate carboxylase]

The regulation of gluconeogenesis will be in direct contrast to the regulation of glycolysis. In general, negative effectors of glycolysis are positive effectors of gluconeogenesis. Regulation of the activity of PFK-1 and Fructose 1, 6 - bisphosphatase is the most significant site for controlling the flux toward glucose oxidation or glucose synthesis. As described in control of glycolysis, this is predominantly controlled by fructose-2, 6-bisphosphate, Fructose 2, 6-bisphosphatase which is a powerful negative allosteric effector of Fructose 1, 6-bisphosphatase activity.

The level of Fructose 2, 6-bisphosphatase will decline in hepatocytes in response to glucagon stimulation as well as stimulation by catecholamines. Each of these signals is elicited through activation of cAMP-dependent protein kinase (PKA). One substrate for PKA is PFK-2, the bifunctional enzyme responsible for the synthesis and hydrolysis of F2, 6BP. When PFK-2 is phosphorylated by PKA it acts as a phosphatase leading to the dephosphorylation of Fructose 2, 6-bisphosphatase with a concomitant increase in Fructose 1, 6-bisphosphatase activity and a decrease in PFK-1 activity. Secondarily, Fructose 1, 6- bisphosphatase activity is regulated by the ATP/ADP ratio. When this is high, gluconeogenesis can proceed maximally.



Regulation of glycolysis and gluconeogenesis by fructose 2, 6-bisphosphate (F2, 6BP). The major sites for regulation of glycolysis and gluconeogenesis are the phosphofructokinase-1 (PFK-1) and fructose-1, 6- bisphosphatase (F-1, 6-BPase) catalyzed reactions. PFK-2 is the kinase activity and F-2, 6-BPase is the phosphatase activity of the bi-functional regulatory enzyme, phosphofructokinase-2/fructose-2, and 6- bisphosphatase. PKA is cAMP-dependent protein kinase which phosphorylates PFK-2/F-2, 6-BPase turning on the phosphatase activity. (+ Ve) and (- ve) refer to positive and negative activities, respectively.

3.11.1 Glycogen Metabolism:

- Glycogen is a branched polymer of **α-D-glucose**.
- The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use.
- The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.
- Approximately 400 g of glycogen make up one to two percent of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to ten percent of the fresh weight of a well-fed adult liver.
- ***Structure of Glycogen:*** Glycogen is a branched-chain homopolysaccharide made exclusively from α-D-glucose. The primary glycosidic bond is α **(1→4)** linkage. After an average of eight–ten glucosyl residues, there is a branch containing α **(1→6)** linkage. A single molecule of glycogen can have a molecular mass of up to 10^8 daltons.
- Liver glycogen stores increase during the well-fed state, and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks).

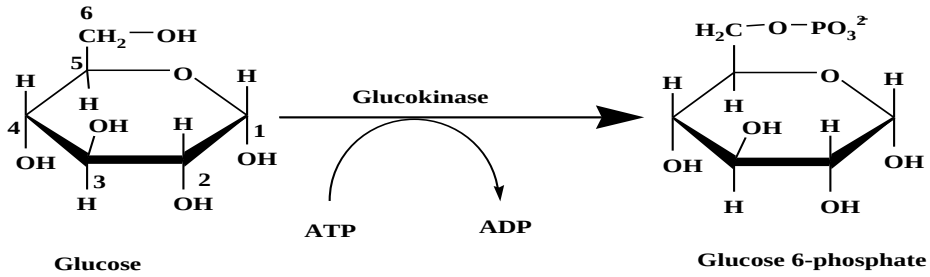
3.11.2 Glycogen Biosynthesis:

- The process of biosynthesis of glycogen from glucose is known as glycogenesis.
- The process occurs in the cytosol, and requires energy supplied by ATP for the phosphorylation of glucose and uridine triphosphate (UTP).
- Glycogenesis is a very essential process since the excess of glucose is converted and stored up as glycogen which could be utilised at the time of requirement. In the absence of this process the tissues are exposed to excess of glucose immediately after a meal and they are starved of it at other times.
- The following are the various reactions of glycogenesis are as follows:

Step 1:

Glucose is phosphorylated to glucose 6-phosphate, a reaction that is common to the first reaction in the pathway of glycolysis from glucose.

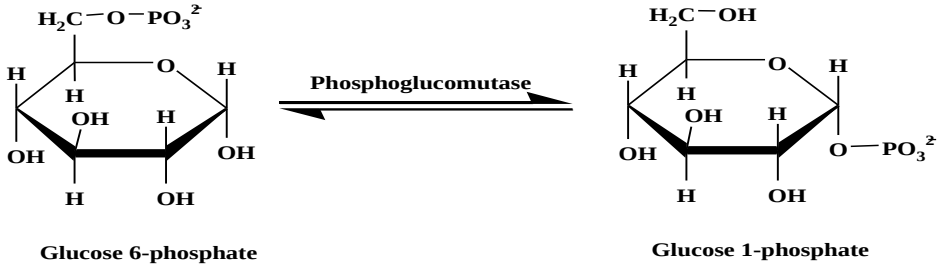
This reaction is catalysed by hexokinase in muscle and glucokinase in liver in the presence of ATP.



Step 2:

Glucose 6-phosphate is then reversibly converted to glucose 1-phosphate in a reaction catalysed by enzyme phosphogluco mutase.

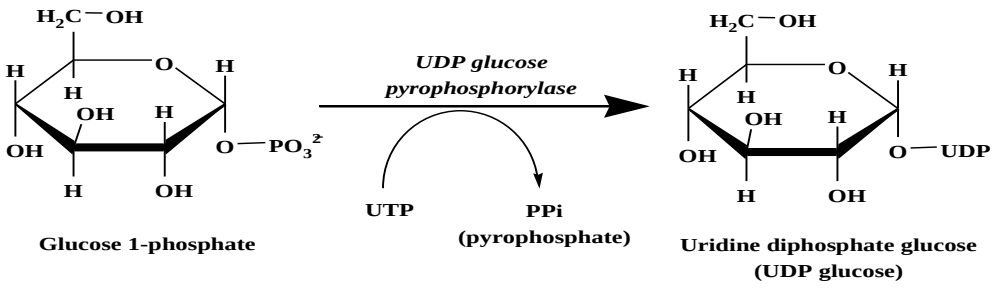
This process requires Mg^{2+} and a small amount of glucose 1, 6-diphosphate as coenzyme.



Step 3:

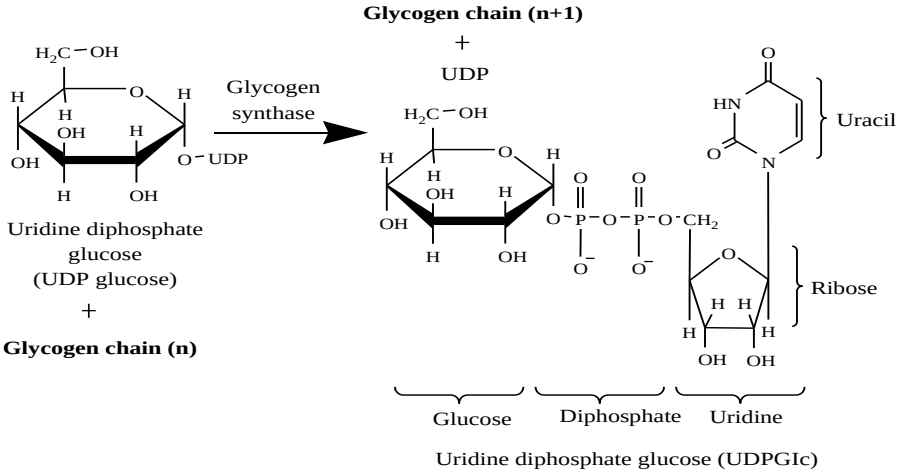
The glucose 1-phosphate is then activated by the energy produced by the hydrolysis of uridine triphosphate (UTP) in the presence of uridine diphosphate glucose pyrophophosrylase.

This is a key reaction in glycogen biosynthesis.



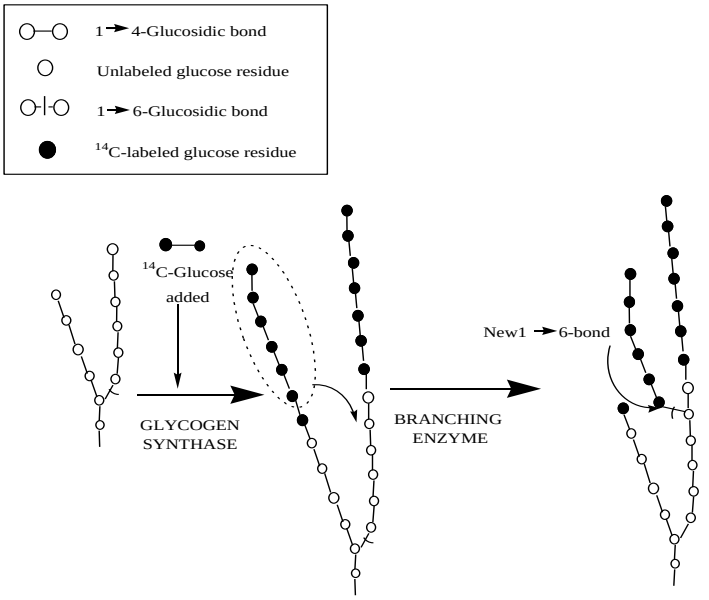
Step 4:

UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by *glycogen synthase*, which promotes the transfer of the glucose residue from UDP-glucose to a nonreducing end of a branched glycogen chain.



Step 5:

When the chain has become long with more than 8 glucose units, a second enzyme, namely branching enzyme ***amylo 1-4 to 1-6 transglycosylase*** acts on the glycogen and helps in joining of 1, 4 glycogen chains with a similar neighboruing chain to form α-1-6-linkage, thus form a branching point in the molecule. Glycogen thus formed may be stored in liver, muscles and tissues.



If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) molecule of glucosyl residues attached by α (1→4) linkages. Such a compound is found in plant tissues, and is called **amylose**.

In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added.

3.11.3 Degradation of Glycogen (Glycogenolysis):

- When the blood sugar level falls (Hypoglycemia), glycogen stored in the tissues especially glycogen of liver and muscles may be broken down and this process of breakdown of glycogen is called glycogenolysis.
- When glycogen is degraded, the primary product is glucose 1-phosphate, obtained by breaking α (1→4) glycosidic bond. In addition, free glucose is released from each α (1→6)-linked glucosyl residue.

a. Shortening of Chains:

- Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis until four glucosyl units remain on each chain before a branch point.

The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further.

b. Removal of Branches:

- Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme. First, oligo-α (1→4) →α (1→4)-glucan transferase removes the outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, α (1→4) bond is broken and α (1→4) bond is made, and the enzyme functions as a 4:4 transferase.
- Then the remaining single glucose residue attached in and α (1→6) linkage is removed hydrolytically by amylo-α (1→6)-glucosidase activity, releasing free glucose.
- The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units from the next branch are reached.

c. Conversion of Glucose 1-Phosphate to Glucose 6-Phosphate:

- Glucose 1-phosphate, produced by glycogen phosphorylase, is converted in the cytosol to glucose 6- phosphate by phosphoglucomutase.
- In the liver, glucose 6-phosphate is translocated into the endoplasmic reticulum (ER) by glucose 6- phosphate translocase. There it is converted to glucose by

glucose 6-phosphatase—the same enzyme used in the last step of gluconeogenesis.

- In the muscle, glucose 6-phosphate cannot be dephosphorylated because of a lack of glucose 6- phosphatase. Instead, it enters glycolysis, providing energy needed for muscle contraction.

d. Lysosomal Degradation of Glycogen:

- A small amount (one–three percent) of glycogen is continuously degraded by the lysosomal enzyme, α (1→4)-glucosidase (acid maltase). The purpose of this pathway is unknown.
- However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease type II (Pompe disease).

> ***Note By:*** Glycogen storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to decreased degradation.

3.12 Glycogen Storage Diseases (GSD):

- Glycogen storage disease (GSD, also glycogenosis and dextrinosis) Glycogen storage disease (GSD, also glycogenosis and dextrinosis) is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types
- Glycogen is a major source of energy for the body. It is stored in the form of glycogen in both the liver and muscles and later released with the help of enzymes. Persons affected by GSD have an inherited defect in one of the enzymes responsible for forming or releasing glycogen as it is needed by the body during exercise and/or between meals.

Types of Glycogen Storage Disease

3.12.1 Type 0 - Glycogen Synthase Deficiency:

The enzyme glycogen synthase is needed for the body to make glycogen. A deficiency results in very low amounts of glycogen stored in the liver. A person between meals can develop very low blood sugar levels, known as hypoglycemia.

3.12.2 Type I - Von Gierke Disease:

It is also known as glucose-6-phosphatase deficiency, in which the body cannot break down glycogen for energy.

- Gycogen is stored in the liver and muscles and is normally broken down into glucose when you do not eat. It occurs when the body lacks the protein (enzyme) that

releases glucose from glycogen. This causes abnormal amounts of glycogen to build up in certain tissues. When glycogen is not broken down properly, it leads to low blood sugar.

- Von Gierke disease is inherited, which means it is passed down through families. If both parents carry the defective gene related to this condition, each of their children **has a 25% chance of developing the disease.**

3.12.3 Glycogen Storage Disease Type II:

- It is also known as Pompe disease or acid maltase deficiency.
- It is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body's cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally.
- Three types of Pompe disease,

i. The classic form of infantile-onset Pompe disease begins within a few months of birth. Infants with this disorder typically experience muscle weakness (myopathy), poor muscle tone (hypotonia), an enlarged liver (hepatomegaly), and heart defects. Affected infants may also fail to gain weight and grow at the expected rate (failure to thrive) and have breathing problems. If untreated, this form of Pompe disease leads to death from heart failure in the first year of life.

ii. The non-classic form of infantile-onset Pompe disease usually appears by age 1. It is characterized by delayed motor skills (such as rolling over and sitting) and progressive muscle weakness. The heart may be abnormally large (cardiomegaly), but affected individuals usually do not experience heart failure. The muscle weakness in this disorder leads to serious breathing problems, and most children with non-classic infantile-onset Pompe disease live only into early childhood.

iii. The late-onset type of Pompe disease may not become apparent until later in childhood, adolescence, or adulthood. Late-onset Pompe disease is usually milder than the infantile-onset forms of this disorder and is less likely to involve the heart. As the disorder progresses, breathing problems can lead to respiratory failure take place.

3.12 4 Glycogen Storage Disease Type IV:

- It is also known as *Andersen disease* or *brancher enzyme* deficiency.
- Deficient activity of the glycogen-branching enzyme is the cause of GSD Type IV. It results in accumulation of abnormal glycogen in the liver, muscle and other tissues.

3.12.5 Glycogen Storage Disease Type V:

- It is also known as *McArdle Disease.*
- It cause due to *myophosphorylase* deficiency.
- It is a rare metabolic disorder which causes muscle pain in everyday activities and exercise. If activity is prolonged despite the pain then muscle damage ensues with the risk of muscle breakdown and kidney failure.

Note by:

Myophosphorylase is the muscle isoform of the enzyme *glycogen phosphorylase*. This enzyme helps break down glycogen (a form of stored carbohydrate) into glucose-1-phosphate, (not glucose) so that it can be utilized within the muscle cell.

3.12.6 Glycogen Storage Disease Type VI:

- It is also known as hers disease.
- It cause due to liver phosphorylase deficiency.
- Note by: liver phosphorylase is an enzyme that catalyzes the breakdown of liver glycogen to glucose-1- phosphate.

3.12.7 Glycogen Storage Disease Type VII:

- It is also known as Tarui disease.
- It cause due to muscle phosphofructokinase deficiency.
- The phosphofructokinase enzyme which is needed to facilitate the breakdown of glycogen into energy in muscle. This results in reduced amount of energy available to muscles during exercise.

- The body breaks down muscle when trying to attain energy, which causes symptoms such as muscle pain, cramping, fatigue and tenderness. With the breakdown of muscle and the release of the red protein myoglobin, red-brown urine may be seen.
- The enzyme deficiency is due to abnormalities in the muscle phosphofructokinase gene. GSD VII is inherited as an autosomal recessive genetic disorder.

3.12.8 Glycogen Storage Disease Type VIII:

- It cause due to *liver glycogen phosphorylase kinase* deficiency**.**
- In most individuals apart from liver enlargement there are few other problems. There is usually no tendency to low blood sugar, the liver becomes smaller with age and children grow normally.

Summary of Glycogen storage disease (GSD, also glycogenosis and dextrinosis)

Glycogensis	Name	Cause of Disorder	Characteristics
Type – I	Von Gierke's disease	Deficiency of glucose-6phosphatase	Liver cell and renal tubule cell loaded with glycogen, Hypoglycemia, lactic acidemia, ketosis, and hyperlipemia.

Glycogensis	Name	Cause of Disorder	Characteristics
Type – II	Pompe's disease	Deficiency of lysosomal ά-1→4 and 1→6-glucosidase (acid maltase)	Fatal, accumulation of glycogen in lysosomes, heart failure.
Type – III	Limit dextrainosis, Forbes' or Cori's disease	Absence of debranching enzyme	Accumulation of a characteristic branched polysaccharide.
Type – IV	Amlopectinosis, Andersen's disease	Absence of branching enzyme	Accumulation of a plysaccharide having few branch points. Death due to cardiac or liver failure in first year of life.
Type – V	Myophosphorylase deficiency, McArdle's syndrome	Absence of muscle phosphorylase	Dmiminished exercise tolerance, muscles have abnomally high glycogen content (2.5-4.1%) little or no lactate in blood after excercise.
Type – VI	Hers' disease	Deficiency of liver phosphorylase	High glycogen content in liver tendency toward hypoglycemia.
Type – VII	Tarui's disease	Deficiency of phosphofructokinase in muscle and erythrocytes	As for type V but also possibility of the molytic anemia
Type – VIII		Deficiency of liver phosphorylase kinase	As for type VI.

3.13 Hormone Control of Carbohydrate Metabolism:

The metabolism of carbohydrates is regulated by a variety of hormones and other molecules. Some of these have already been mentioned in previous sections. The proper functions of the body are dependent on precise control of the glucose concentration in the blood. The normal fasting level of glucose in the blood is 70-90 mg/100 ml.

If the concentration of glucose in blood is too high (above 120 mg/100 mL) a condition known as hyperglycemia results. Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates.

If the concentration of glucose is too low (below 70 mg/100 ml) a condition of hypoglycemia exists. Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse perspiration, rapid heartbeat, and possible loss of consciousness.

Insulin:

Insulin, a polypeptide, is secreted from the pancreas in response to a hyperglycemia condition which usually results shortly after ingesting a meal.

The major effect of insulin is to promote the transport of sugar across the cell membrane of fat and muscle cells. In addition, insulin promotes anabolic processes such as increasing the rate of synthesis for glycogen (glycogenesis), fatty acids, and proteins. Insulin inhibits the catabolic processes such as the breakdown of glycogen and fat.

A deficiency of insulin (hypoinsulinism) results in a permanent hyperglycemic condition known as diabetes mellitus. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood. Fatty acid metabolism is also upset. For this reason, a detailed study of diabetes mellitus must wait until the next chapter.

Hyperinsulinism (too much insulin) leads to the hypoglycemic condition. Excessive amounts of glucose are removed from the blood. Severe hypoglycemia may result when a diabetic injects too much insulin. A severe insulin shock may result in a coma since glucose does not reach the brain.

A diabetic usually carries a glucose rich food, such as candy, to provide a quick supply of glucose to replenish depleted glucose levels caused by too much insulin. sA functional type of hypoglycemia results in some individuals from an over stimulation of insulin.

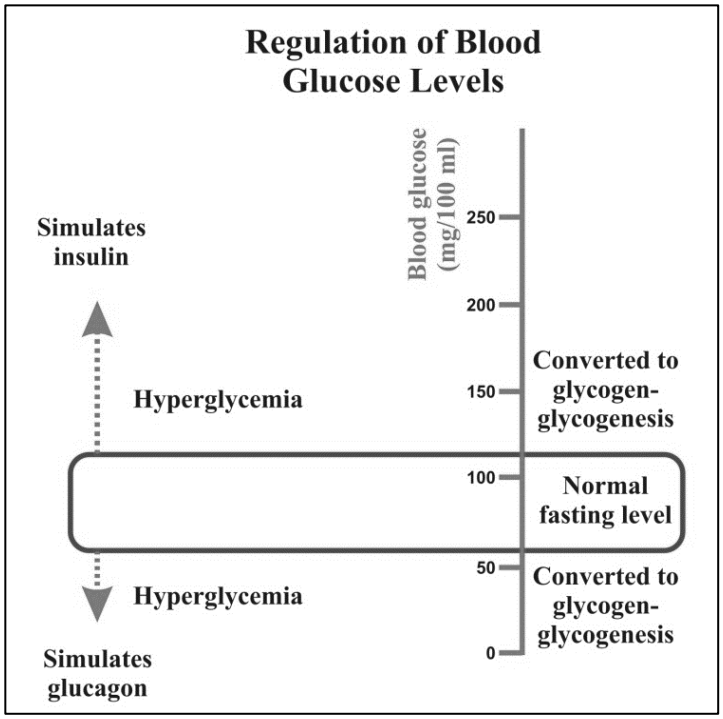
The causes of hypoglycemia are not completely understood, but it occurs in some people after eating heavily sugared food such as heavily sugared cereal and/or coffee and sweet rolls. The initial high glucose levels over stimulates the pancreas to produce too much insulin.

The excess insulin causes blood sugar levels to drop below normal after 2-3 hours which may cause the person to feel sleepy, irritable, and generally tired. The condition is only exacerbated by a "quick fix" of more sweetened coffee, pastry, or candy since more insulin is produced again. A protein rich breakfast would correct the condition by allowing glucose to enter the blood stream more slowly.

Glucagon:

If one hormone, insulin, controls the excess of glucose in the blood by stimulating synthesis of glycogen, then other hormones must respond to low levels of glucose. The liver is more responsive to **glucagon**, a peptide also secreted by the pancreas.

Glucagon increases glucose levels in the blood by stimulating the breakdown of glycogen (glycogenolysis) in the liver into glucose which leaves the liver cells and enters the blood stream. The method of hormone stimulation is a complex cascade effect. The exact sequence has been worked out in the most detail for epinephrine (adrenalin) although glucagon works in a similar fashion.



3.14 Chapter at a Glance:

Term	Description
CoA	Coenzyme A
TCA	Tricarboxilic acid cycle
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non-insulin dependent diabetes mellitus
GTT	Glucose tolerance test
SGPT	Serum glutamic pyruvic transaminase

3.15 Exercises:

3.15.1 Multiple choice Questions:

1. Name the pathway for glucose synthesis by non-carbohydrate precursors?

(a) Glycogenesis
(b) Glycolysis
(c) Gluconeogenesis
(d) Glycogenolysis

2. What is the site for gluconeogenesis?

   (a) Liver (b) Blood
   (c) Muscles (d) Brain

3. Which of the following is not the precursor of gluconeogenesis?

   (a) Glycolytic products (b) Citric acid cycle intermediates
   (c) Glucogenic amino acid (d) Lysine or leucine

4. Name the enzyme which is responsible for the conversion of pyruvate to phosphoenolpyruvate (PEP)?

   (a) Pyruvate carboxylase (b) Pyruvate carboxykinase
   (c) Glucose 6-phosphatase (d) Phosphofructokinase

5. Which of the following are major sites for glycogen storage?

   (a) Adipose tissue (b) Bones
   (c) Muscle and liver (d) Kidney and liver

6. Which of the following is the precursor of glycogen?

   (a) Glycerol 3-phosphate (b) Malate
   (c) UDP-glucose (d) Leucine and lysine

7. The priming function in glycogen synthesis is carried out by_____

   (a) Lysine (b) Arginine
   (c) Glycogenin (d) Glutamate

8. Name the enzyme which is used for branching of glycogen?

   (a) Branching enzyme (b) Hexokinase
   (c) Phosphoglucomutase (d) Glycogen synthase

9. Which of the following hormone maintain blood glucose level by activation of gluconeogenesis?

   (a) Nor-epinephrine (b) Glucagon
   (c) Insulin (d) Epinephrine

10. Name the hormone which is secreted in an emergency or in stress condition?

    (a) Epinephrine (b) Glucagon
    (c) Insulin (d) Melanin

3.15.2 Short Answer Questions:

1. Y. Write a note on atherosclerosis,
2. Y. Write a note on fatty liver.
3. Y. Write a note on obesity.
4. Y. Give the account of steroid hormone
5. Y. Give the account of synthesis of vitamin D

3.15.3 Long Answer Questions:

1. Z. Write a note on β-Oxidation of saturated fatty acid.
2. Z. Give Formation and utilization of ketone bodies; ketoacidosis.
3. Z. Give the Biological significance of cholesterol.
4. Z Give the detailed account for the conversion of cholesterol into bile acids.
5. Z. Write a note on Hypercholesterolemia,

Answer key MCQs:

(1) – (c), (2) – (a), (3) – (d), (4) – (b), (5) – (c), (6) – (c), (7) – (c), (8) – (a), (9) – (b), (10) – (a).

3.16 Bibliography:

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Chapter 4

Nucleic Acid Metabolism and Genetic Information

4.1 Introduction:

Purines and pyrimidines are the two families of nitrogenous bases that make up nucleic acids – in other words, they are the building blocks of DNA and RNA.

I. Structures and Functions of Nucleotides:

A. A nucleoside is formed from the linkage of a sugar with a nitrogen-containing base.

i. The bases that make up the physiologically relevant nucleosides all have ring structures.
   - The purines adenine, guanine, and inosine have a double-ring system.
   - The pyrimidines cytosine, thymine, and uracil have six-membered ring structures.

ii. Ribose and 2-deoxyribose are the main sugars found in nucleosides and nucleotides.

B. Nucleotides are nucleosides to which one, two, or three phosphate groups have been added to the sugar.

i. The bonds that interconnect the phosphate groups yield high energy when they are broken or hydrolyzed.
   - ATP is the main form for provision of energy to make these bonds.
   - Hydrolysis of these bonds is often used to drive biosynthetic reactions.

ii. Many coenzymes are AMP or ADP derivatives, including NAD+, NADP+, FAD, and coenzyme A.

iii. ATP and sometimes GTP can serve as a phosphate donor in many physiologic reactions because they have a high phosphoryl transfer potential.

C. Nucleotides have many important functions in the body.

i. Nucleotides serve as the building blocks for synthesis of the nucleic acids DNA and RNA.

ii. Nucleotides are components of several coenzyme structures and of ATP.

iii. Nucleotides control rates of many enzyme-catalyzed reactions by feedback and allosteric regulation.

iv. In cyclic forms, such as cyclic AMP and cyclic GMP, nucleotides serve critical roles in cellular signaling mechanisms.

II. Biosynthesis of Purines: There are two pathways by which nucleotides are made available for the formation of nucleic acids:

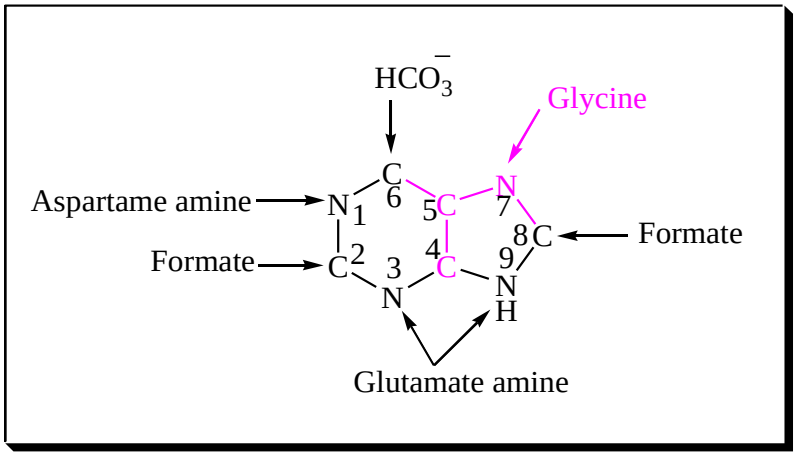
i. Denovo synthesis i.e. new synthesis and
ii. Salvage process i.e. recycling of the bases.

4.2 Synthesis of Purine a New:

De novo (all over again) synthesis of purine nucleotides is synthesis of purines a new. The purine ring is synthesized along with the nucleotide i.e. attached to the ribose sugar provided from HMP pathway. This pathway supplies ribose sugar for the formation of the nucleotide. Activated form of D-ribose-5-phosphate serves as the starting material on which purine ring is build up step by step.

4.3 Precursors of the Members of Purine Ring are:

i. N-1 is contributed by nitrogen of aspartate.
ii. N-3 and N-9 arise from amide nitrogen of glutamine.
iii. C-2 and C-8 originate from the formate.
iv. C-6 is embedded from respiratory carbon dioxide.
v. C-4, C-5 and N-7 are taken up from glycine.



A. The cell maintains an important pool of purine nucleotides for synthesis of coenzymes and precursors for DNA and RNA and to support reactions that are coupled to ATP hydrolysis.
B. Purine nucleotides can be synthesized de novo from amphibolic or dual-purpose intermediates, which may be derived either from anabolic or catabolic pathways.
C. Ribose 5-phosphate derived from the pentose phosphate pathway or from dietary sources is the starting material that eventually gives rise to inosine monophosphate (IMP).
D. The overall strategy is to build the carbon-nitrogen skeleton of a purine ring system in a 12-step process directly on the sugar-phosphate starting material.

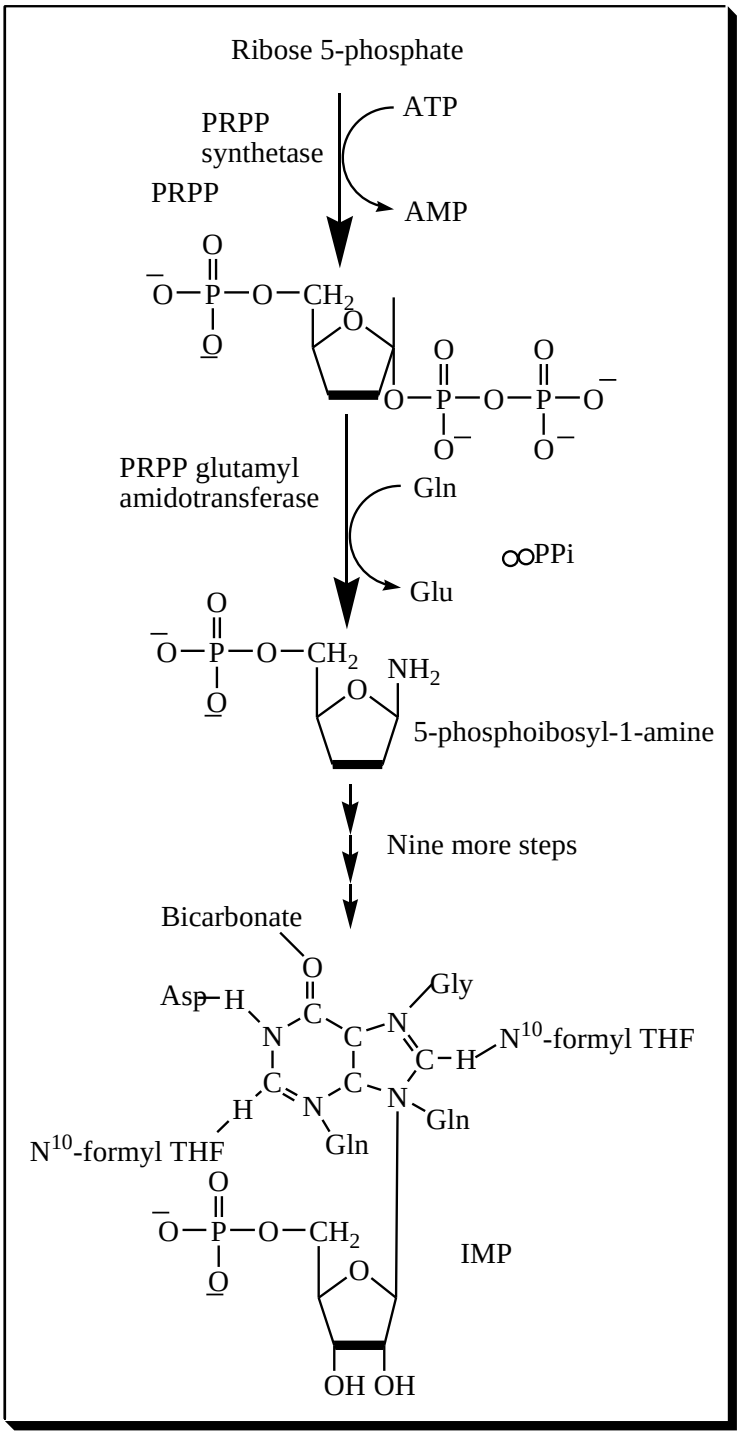


Figure 4.1: Overview of purine synthesis. Details of the first two reaction and sources of the atoms of the purine ring in inosline 5'monophosphate (IMP) are shown. PRPP, 5'-Phosphoribosy-I-pyrophosphate: Gln, glutamine: Gly, glycine: Asp, aspartate: THF, tetrahydrofa-late.

a. The first step creates the multi-purpose intermediate 5'-phosphoribosyl-1-pyrophosphate (PRPP).
b. Then, PRPP glutamyl amidotransferase, the key regulatory enzyme, acts upon PRPP to begin making the purine ring; this is the committed step of purine synthesis.

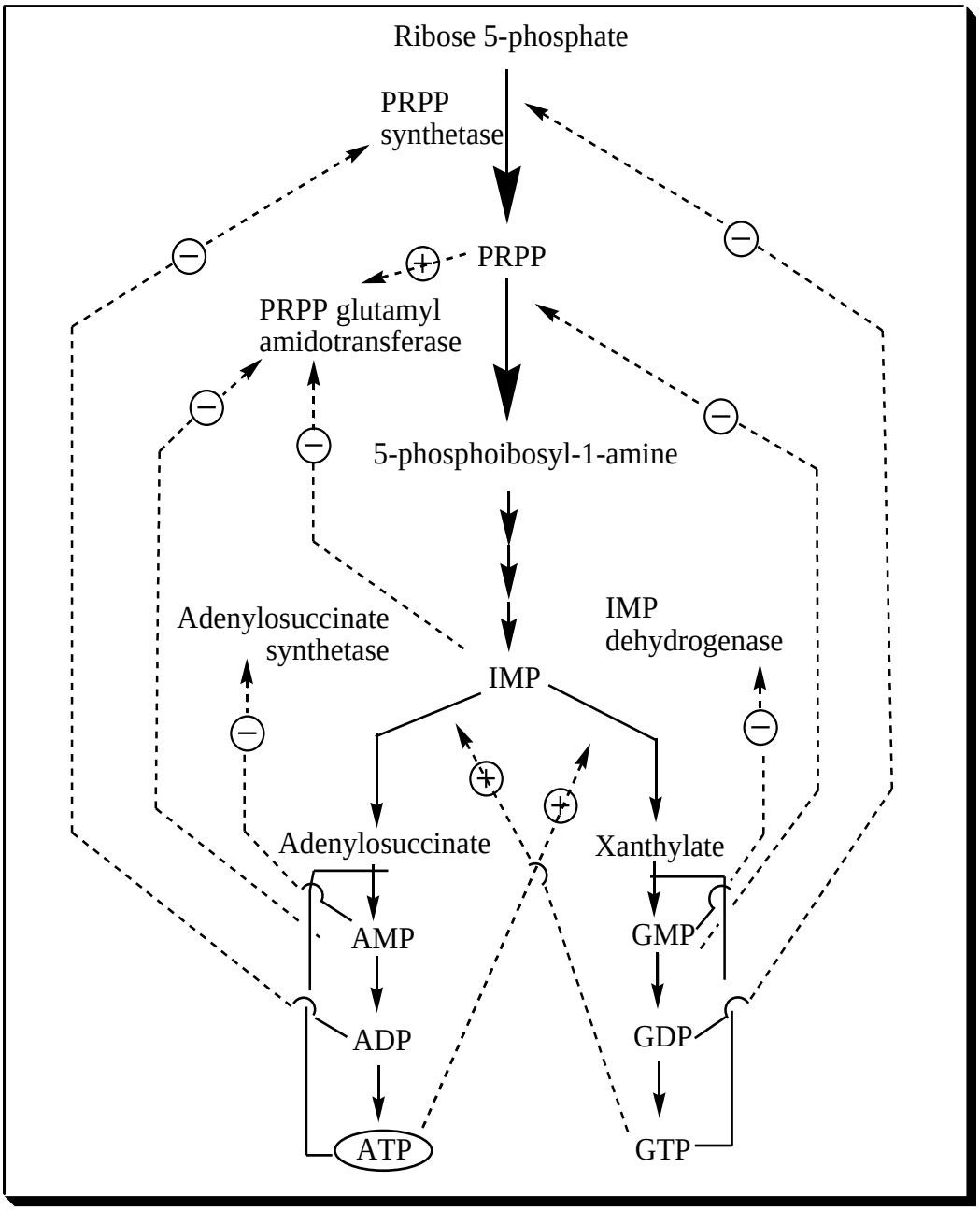


Figure 4.2: Regulation of purine synthesis by the nucleotides and the intermediate, 5'-phosphoribosy-I-pyrophosphate (PRPP). Both feedback and feed-forward mechanims are utilized in the intricate scheme. IMP, inosine moniphosphate

c. Carbons are added to the growing ring in several ways:

i. By one-carbon transfer by enzymes that use tetrahydrofolate (THF) coenzymes.
ii. By incorporation of glycine in the structure.
iii. By addition of CO_2 in the form of bicarbonate.

d. Nitrogens are added by the following:

i. Aminotransfer reactions with glutamine as donor.
ii. In a two-step mechanism with aspartic acid as donor.

e. Conversion of the main product of de novo synthesis, IMP, to GMP or AMP, occurs in two reactions, both of which are inhibited by feedback regulation by the end products.
f. Overall flux through the purine synthetic pathways is regulated primarily by feedback inhibition of PRPP glutamyl amidotransferase, by IMP, AMP, and GMP (Figure 4.2).

4.4 Folic Acid Deficiency:

- Decreased levels of folate coenzymes needed for various reactions of de novo purine synthesis and thymine synthesis produce shortages of deoxyribonucleotides and consequent impaired DNA synthesis in many tissues.
- Blood levels of folic acid may become inadequate due to dietary insufficiency or poor absorption due to intestinal problems or alcoholism. Folate coenzyme concentrations may also decline as a result of treatment with drugs that inhibit dihydrofolate reductase, eg, methotrexate.
- Patients with folic acid deficiency may have diarrhea and nausea, but the principal symptoms are weakness and easy fatigability due to megaloblastic anemia arising from impaired cell division in the bone marrow.
- Folate deficiency during pregnancy is a major contributor to neural tube defects because of the critical role of folate in neuronal development.
- Folate supplementation of food in the United States is expected to reduce folate-associated birth defects by up to 70%.

E. Formation of deoxyribonucleotides by reduction of the 2′-hydroxyl group of the ribose sugars on the ribonucleoside diphosphates ADP and GDP is catalyzed by ribonucleotide reductase (Figure 4.3).

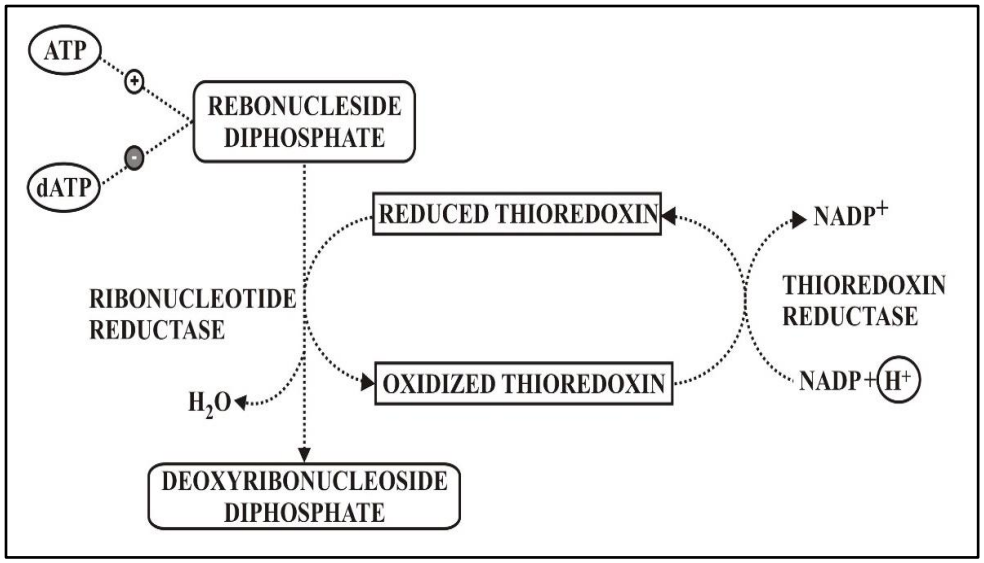


Figure 4.3: Conversion of Ribonucleosides to deoxyribonucleosides by ribonucleoside reductase.

a. Thioredoxin serves as an electron donor for the reduction.
b. The enzyme is also responsible for converting cytidine diphosphate (CDP) to 2′-dCDP and uridine diphosphate (UDP) to 2′-dUDP for use in making nucleotides for DNA synthesis.
c. Regulation of ribonucleotide reductase by both positive feedback from ATP and negative feedback by various 2′-deoxynucleoside triphosphates (eg, dATP) is tightly coupled to the need for DNA synthesis.

III. Biosynthesis of Pyrimidines:

The pyrimidine ring is synthesized first and is then attached to ribose 5-phosphate to eventually produce the nucleotide uridine 5′-monophosphate (UMP).

A. The first step in the pathway is synthesis of carbamoyl phosphate (Figure 4.4)

- An ammonium ion contributed by glutamine is combined with bicarbonate (derived from dissolved CO_2) in a two-step reaction that requires hydrolysis of two molecules of ATP.
- This complex reaction is catalyzed by carbamoyl phosphate synthetase II (CPS-II).
- CPS-II, the critical enzyme regulating the pyrimidine synthetic pathway, is activated by ATP and PRPP and feedback-inhibited by the end product UTP.
- CPS-II, a cytosolic enzyme, is different from the mitochondrial enzyme of the urea cycle CPS-I.

B. Condensation of carbamoyl phosphate with aspartate brings together all the atoms needed to make the main pyrimidine ring (Figure 4.4)

C. Closure of the ring followed by a reduction step leads to formation of the pyrimidine base orotate.

D. Orotate is then connected to ribose 5-phosphate and decarboxylated to produce UMP.

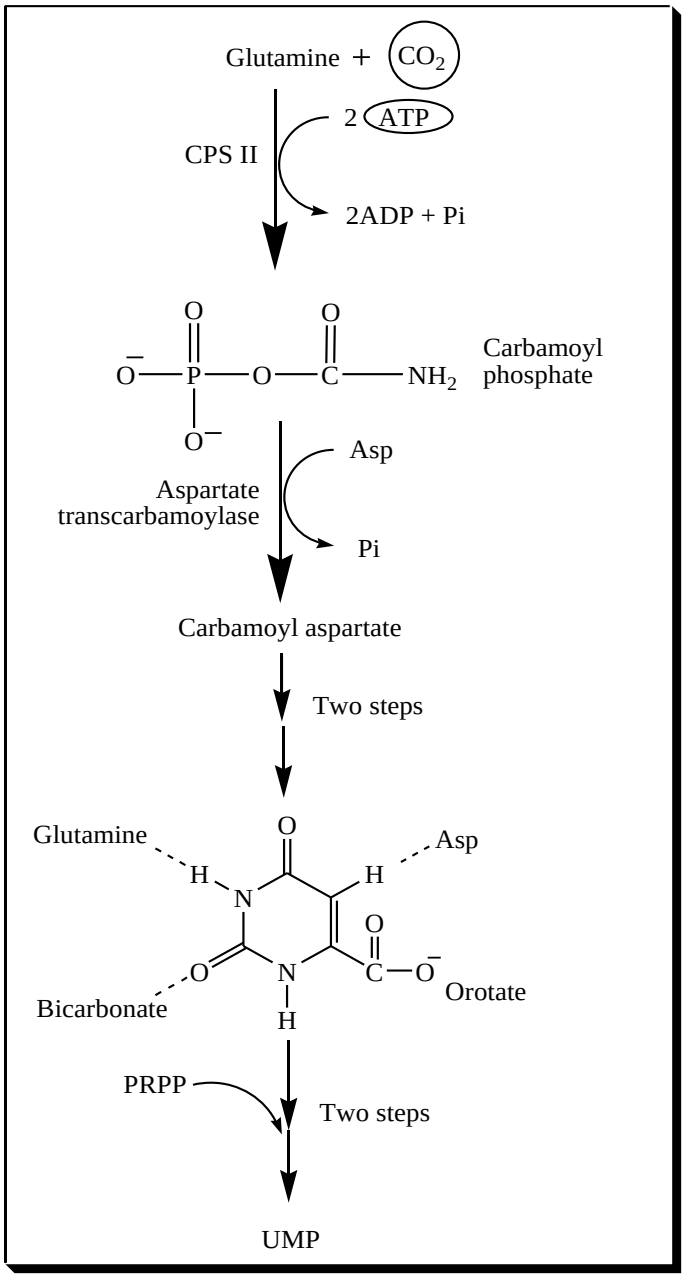


Figure 4.4: Overview of pyrimidine synthesis. The free base is synthesized beginning with condensation of bicarbonate and an ammonuim ion derived from glutamine. CPS-II, carbamoyl posphate synthetase: Asp, aspartate: PRPP,5'-phosphoribosyl –I-pyrophosphate: UMP, uridine moniohos-phare.

4.5 Purine Catabolism:

The end product of purine catabolism in man is uric acid. Other mammals have the enzyme urate oxidase and excrete the more soluble allantoin as the end product.

Man does not have this enzyme so urate is the end product for us. Uric acid is formed primarily in the liver and excreted by the kidney into the urine.

Purines are catabolized to xanthine and uric acid in human. Uric acid then is secreted in urine.

- Gout is an arthritis that has hyperuricemia.
- Lesch-Nyhan syndrome and Von Gierke disease are disorder of this purine catabolism.

4.6 Nucleotides to Bases:

Guanine nucleotides are hydrolyzed to the nucleoside guanosine which undergoes phosphorolysis to guanine and ribose 1-P.

Man's intracellular nucleotidases are not very active toward AMP, however.

Rather, AMP is deaminated by the enzyme adenylate (AMP) deaminase to IMP.

In the catobilsm of purine nucleotides, IMP is further degraded by hydrolysis with nucleotidase to inosine and then phosphorolysis to hypoxanthine.

Adenosine does occur but usually arises from *S*-Adenosylmethionine during the course of transmethylation reactions. Adenosine is deaminated to inosine by an adenosine deaminase.

Deficiencies in either adenosine deaminase or in the purine nucleoside phosphorylase lead to two different immunodeficiency diseases by mechanisms that are not clearly understood.

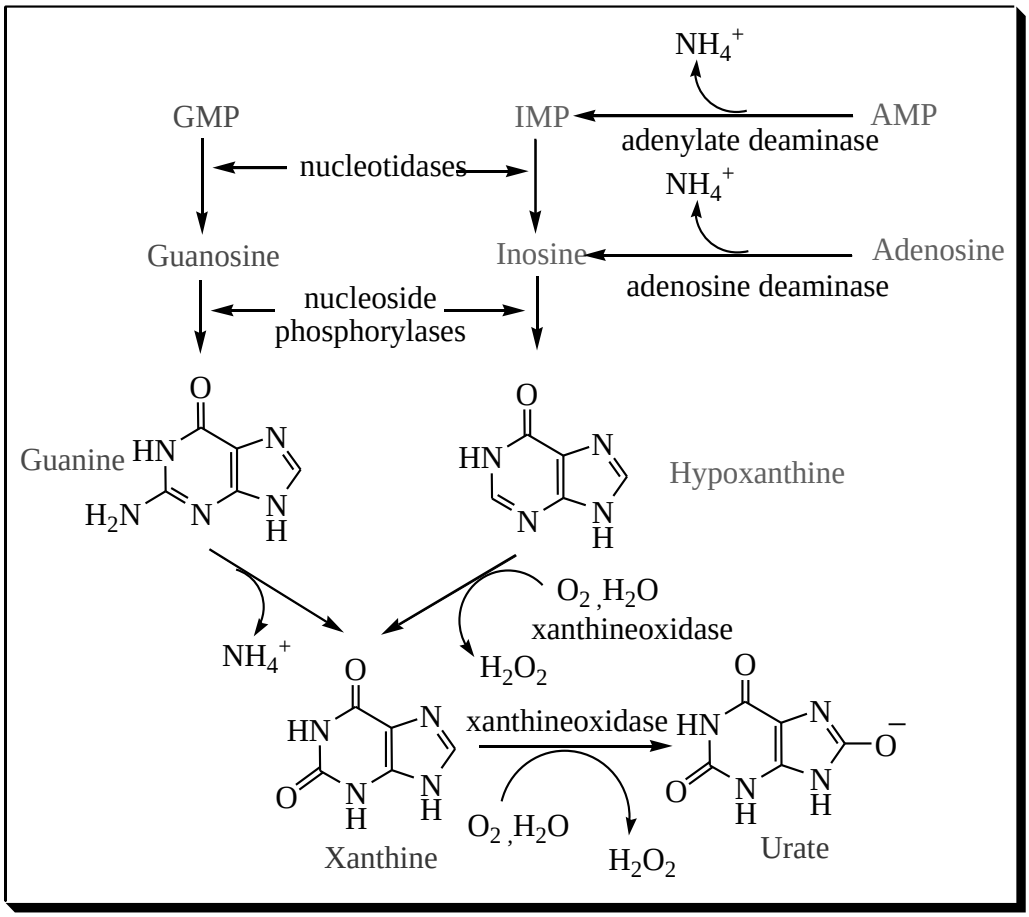
With adenosine deaminase deficiency, both T and B-cell immunity is affected. The phosphorylase deficiency affects the T cells but B cells are normal.

In September, 1990, a 4 year old girl was treated for adenosine deaminase deficiency by genetically engineering her cells to incorporate the gene.

The treatment, so far, seems to be successful. Whether or not methylated purines are catabolized depends upon the location of the methyl group.

If the methyl is on an $-NH_2$, it is removed along with the $-NH_2$ and the core is metabolized in the usual fashion.

If the methyl is on ring nitrogen, the compound is excreted unchanged in the urine.



4.7 Bases to Uric Acid:

Both adenine and guanine nucleotides converge at the common intermediate xanthine. Hypoxanthine, representing the original adenine, is oxidized to xanthine by the enzyme xanthine oxidase. Guanine is deaminated, with the amino group released as ammonia, to xanthine. If this process is occurring in tissues other than liver, most of the ammonia will be transported to the liver as glutamine for ultimate excretion as urea.

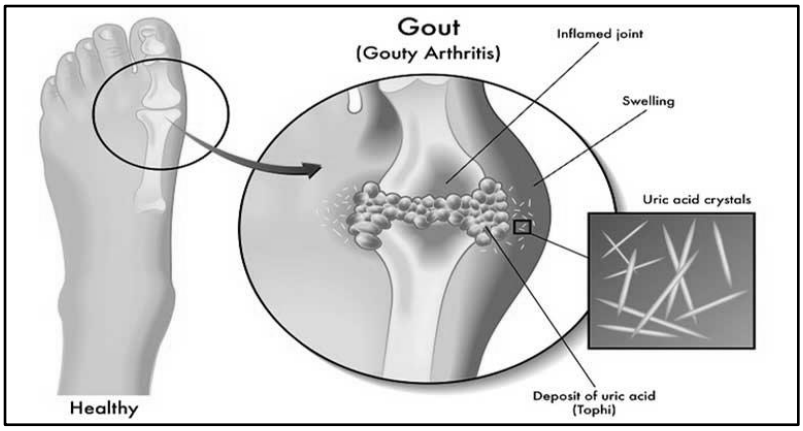
Xanthine, like hypoxanthine, is oxidized by oxygen and xanthine oxidase with the production of hydrogen peroxide. In man, the urate is excreted and the hydrogen peroxide is degraded by catalase. Xanthine oxidase is present in significant concentration only in liver and intestine. The pathway to the nucleosides, possibly to the free bases, is present in many tissues.

4.8 Gout and Hyperuricemia:

Gout is characterized by painful joint inflammation, most commonly in the first metatarsophalangeal joint, resulting from precipitation of monosodium urate crystals in a joint space.

Diagnosis may be confirmed by identification of monosodium urate crystals in synovial fluid of the affected joint. Gout results from the precipitation of monosodium urate crystals in a joint space. Crystal deposition then triggers immune activation with the release of several inflammatory cytokines and neutrophil recruitment. Over time, the joint space can be irreversibly damaged, leading to chronic pain and disability with grossly deformed joints. Tophi (i.e., subcutaneous nodules comprised of monosodium urate crystals in a matrix of lipids, protein, and mucopolysaccharides) may also form at the joint space. The first metatarsophalangeal joint is most commonly affected. Other common sites include the midtarsal joints, ankles, knees, fingers, wrists, and elbows. Urate crystals may also be deposited throughout the body (e.g., vertebrae, skin, soft tissues), mimicking other disease states. Urate in the blood could accumulate either through an overproduction and/or an underexcretion of uric acid. In gouts caused by an overproduction of uric acid, the defects are in the control mechanisms governing the production of - not uric acid itself - but of the nucleotide precursors. The only major control of urate production that we know so far is the availability of substrates (nucleotides, nucleosides or free bases).

Gout is the most common inflammatory arthropathy, affecting more than 8 million Americans. Gout accounts for approximately 7 million ambulatory visits in the United States annually at a cost of nearly $1 billion. Risk factors include genetics, age, sex, and diet. These factors may contribute to a high serum uric acid level, which is currently defined as a value of at least 6.8 mg per dL (405 μmol per L). Gout is a group of pathological conditions associated with markedly elevated levels of urate in the blood (3-7 mg/dl normal). Hyperuricemia is not always symptomatic, but, in certain individuals, something triggers the deposition of sodium urate crystals in joints and tissues. In addition to the extreme pain accompanying acute attacks, repeated attacks lead to destruction of tissues and severe arthritic-like malformations. The term gout should be restricted to hyperuricemia with the presence of these tophaceous deposits.



4.9 Pathophysiology and Risk Factors:

Genetic mutations may be associated with overproduction—or more often underexcretion—of uric acid because of defects in the renal urate transporter system. The prevalence of gout increases with age and peaks at more than 12% in persons older than 80 years.

Because female sex hormones increase urinary excretion of uric acid, pre-menopausal women have a substantially lower prevalence of gout compared with men (2.0% vs. 5.9%). Black persons have a higher risk. Consuming alcoholic drinks (particularly beer), meat (especially red meat, wild game, and organ meat), some seafood (e.g., shellfish, some large saltwater fish), fruit juice, and beverages sweetened with high-fructose corn syrup increases the risk of gout. Purine-rich foods such as nuts, oatmeal, asparagus, legumes, and mushrooms do not seem to increase the risk. Consumption of dairy products appears to confer slight protection from gout.

A. Prevention:

Serum urate–lowering therapy should be initiated to prevent recurrences in persons with a history of gout and any one of the following: at least two flares per year (one per year in persons with chronic kidney disease stage 2 or greater), tophi, or a history of nephrolithiasis. Serum urate should be lowered to a target of less than 5 to 6 mg per dL (297 to 357 μmol per L), depending on the crystal and tophaceous burden. Normal serum urate levels do not exclude the diagnosis of gout. They should be monitored periodically to assess preventive therapy in patients with recurrent gout and a history of elevated urate levels. Urate-lowering therapy should be continued for three to six months after a flare if there are no ongoing symptoms. Therapy should continue indefinitely if there are ongoing signs or symptoms (e.g., one or more tophi on examination).

B. Dietary Modifications:

Weight gain is a significant risk factor for gout in men, whereas weight loss reduces the risk. Intake of high-fructose corn syrup should be restricted because the fructose contributes to increased uric acid production as a byproduct of adenosine triphosphate catabolism. Patients with gout should limit their intake of purine-rich animal protein (e.g., organ meats, beef, lamb, pork, and shellfish) and avoid alcohol (especially beer). Purine-rich vegetables do not increase the risk of gout. Consumption of vegetables and low-fat or non-fat dairy products should be encouraged.

C. Treatment:

Treatments for chronic hyperuricemia and the resultant gout include the use of uricosuric drugs and drugs that inhibit the production of uric acid. The commonly prescribed uricosuric drug in the US is probenecid. The use of two additional uricosurics, sulfinpyrazone and lesinurad, has been discontinued in the US.

These uricosuric drugs function, at high doses, by competing for urate reuptake from the glomerular filtrate via SLC22A12 and, therefore, reduce renal reabsorption of uric acid allowing for increased excretion in the urine. Acute gout may be treated with nonsteroidal anti-inflammatory drugs, corticosteroids, or colchicine. To reduce the likelihood of recurrent flares, patients should limit their consumption of certain purine-rich foods (e.g., organ meats, shellfish) and avoid alcoholic drinks (especially beer) and beverages sweetened with high-fructose corn syrup. Consumption of vegetables and low-fat or non-fat dairy products should be encouraged.

The use of loop and thiazide diuretics can increase uric acid levels, whereas the use of the angiotensin receptor blocker losartan increases urinary excretion of uric acid. Reduction of uric acid levels is key to avoiding gout flares. Allopurinol and febuxostat are first-line medications for the prevention of recurrent gout, and colchicine and/or probenecid are reserved for patients who cannot tolerate first-line agents or in whom first-line agents are ineffective.

Patients receiving urate-lowering medications should be treated concurrently with nonsteroidal anti-inflammatory drugs, colchicine, or low-dose corticosteroids to prevent flares. Treatment should continue for at least three months after uric acid levels fall below the target goal in those without tophi, and for six months in those with a history of tophi. A more recent xanthine oxidase inhibitor is febuxostat, approved in the US in 2009. Febuxostat is a non-purine analog that functions as a selective non-competitive inhibitor of the active site of xanthine oxidase. Febuxostat is generally only prescribed for patients who do not tolerate allopurinol.

Patients receiving a urate-lowering medication should be treated concurrently with an NSAID, colchicine, or low-dose corticosteroid to prevent a flare. Treatment should continue for at least three months after uric acid levels fall below the target goal in those without tophi, or for six months in those with a history of tophi. NSAIDs and corticosteroids should not be used for long periods without a urate-lowering medication because uric acid crystals continue to accumulate and damage the joint, despite a lack of pain or clinical signs of inflammation. If a patient has gout flare while receiving a urate-lowering agent, the medication should be continued while the flare is treated acutely.

i. Allopurinol: The most commonly prescribed drug for reducing the production of uric acid is the xanthine oxidase inhibitor, allopurinol. Allopurinol is a purine analog that is a structural isomer of hypoxanthine. Another xanthine oxidase inhibitor that was often used if allopurinol failed to lower serum uric acid levels is benzbromarone.

However, benzbromarone is no longer used in the US, having been withdrawn from this market by its manufacturer in 2003. Allopurinol, a xanthine oxidase inhibitor, is a first-line agent to prevent recurrent gout. In patients with gout and chronic kidney disease or congestive heart failure, allopurinol has the added benefit of preventing chronic disease progression. The starting dosage is 100 mg per day, and 300 mg per day is a common maintenance dosage.

ii. Febuxostat: Febuxostat (Uloric) is a xanthine oxidase inhibitor that was approved by the FDA in 2009. Although febuxostat is superior to 300 mg allopurinol at lowering serum uric acid levels, it is not more effective at reducing the frequency of gout flares. Febuxostat is considered a first-line agent to prevent recurrent gout, but it is considerably more expensive than allopurinol.

iii. Colchicine: Colchicine is another treatment option for acute gout. Generic colchicine, which has been used for decades, did not undergo formal review by the U.S. Food and Drug Administration (FDA) for this indication until 2009, when branded colchicine (Colcrys) was approved. However, Colcrys is expensive, and generic colchicine is no longer available.

In addition, colchicine does not have analgesic properties and may be less effective in treating acute flares when given beyond 72 to 96 hours after symptom onset. Common adverse effects include nausea, vomiting, and diarrhea. Colchicine should be used with caution in patients with hepatic or renal impairment.

Colchicine prevents gout flares at a dosage of 0.6 to 1.2 mg per day. The dose should be adjusted in patients with chronic kidney disease and when used with cytochrome P450 3A4 or P-glycoprotein inhibitors. The long-term adverse effects of colchicine include reversible axonal neuromyopathy (less than 1%). Patients should be advised to stop taking colchicine and tell their physician if they experience leg weakness or pain. Treatment should be discontinued if any signs or symptoms of nerve or muscle damage are present. The rare risk of rhabdomyolysis is increased when colchicine is used concomitantly with statins or clarithromycin, especially in older adults or those with chronic kidney disease; therefore, close monitoring is recommended.

iv. Probenecid: Probenecid increases urinary excretion of uric acid and is typically used as a second-line treatment because of numerous drug interactions. Of particular concern, probenecid increases blood levels of methotrexate and ketorolac, which may result in severe toxicity. Probenecid may be used in combination with allopurinol or febuxostat when one drug does not independently lower serum uric acid to target levels. Nephrolithiasis is a common adverse effect that may be avoided by high fluid intake and urine alkalization with potassium citrate.

v. Corticosteroids: Corticosteroids are an appropriate alternative for patients who cannot tolerate NSAIDs or colchicine. Patients with diabetes mellitus can be given corticosteroids for short-term use with appropriate monitoring for hyperglycemia.

When gout is limited to a single joint, intra-articular corticosteroid injection may be preferable to systemic corticosteroids because of their lower adverse effect profile. Rebound flares are common after discontinuation of corticosteroid therapy for acute gout. To reduce the risk of a rebound flare, preventive treatment and initiation of a tapered course of corticosteroids over 10 to 14 days is recommended after resolution of symptoms.

vi. Pegloticase: Pegloticase (Krystexxa) is an intravenous uricase approved by the FDA in 2010. The mechanism of action involves metabolism of uric acid to allantoin. It is a third-line agent and is indicated for treatment of refractory gout. It is usually administered by a rheumatologist and is given every two weeks.

4.10 Lesch-Nyhan Syndrome:

- Lesch-Nyhan syndrome is an X-linked disorder arising from deficiency of HGPRT, which results in failure to salvage hypoxanthine and guanine to the corresponding nucleotides IMP and GMP.
- Inability to utilize PRPP in the salvage pathway leads to PRPP accumulation, which, in conjunction with low levels of IMP and GMP, causes chronic allosteric activation of PRPP glutamyl amidotransferase and excessive purine synthesis.

- The excess purines are degraded to uric acid causing increased blood levels of this metabolite (hyperuricemia) and deposition of sodium urate crystals in the joints and kidneys.
- Patients with Lesch-Nyhan syndrome experience gout-like episodes of joint pain and kidney stones as well as severe neurologic problems, including self-mutilation, spastic movements, and mental retardation. Allopurinol treatment alleviates the symptoms of uric acid overproduction but does not remedy the neurologic problems.

I. Overview of Nucleic Acid Function:

A. DNA is the chemical basis of heredity.

i. DNA comprises the genetic material by which the information to make proteins and RNA is stored and transmitted to offspring.
ii. DNA is a linear polymer of deoxyribonucleotides in which the sequence of purine and pyrimidine bases encodes cellular RNA and protein molecules.
iii. DNA is highly organized into chromosomes, structures that allow the DNA to be packaged tightly for storage in the nucleus of the cell.

a. A diploid human cell contains 46 chromosomes within a 1 μm-diameter nucleus.
b. In order to fit such long molecules in a confined space, the DNA must be compacted.

B. The individual genes of defined sequence in the DNA specify or encode proteins and RNAs needed for all cellular functions.

i. Replication, the process by which copies of the DNA are made, must have very high fidelity or accuracy to ensure proper function of gene products and healthy offspring.
ii. Most errors that occur during replication or as a result of oxidative or chemical damage, termed mutations are repaired before cell division.
iii. When these mutations are not repaired, a heritable change in the DNA occurs.

a. Such a change may alter function or regulation of a gene product in daughter cells upon mitosis.
b. If this occurs in germline cells, the mutation may be passed to offspring through gametes.

iv. Mutations change the sequence of DNA bases and may thus lead to cellular dysfunction or disease.

a. Proteins bearing amino acid substitutions may not attain their correct conformations or properly serve their physiologic functions.
b. Mutations in regulatory areas near genes can prevent proper control over their expression.

C. RNA molecules operate at critical points in many of the processes that involve expression of the information represented in the DNA.

a. Transcription is the process by which RNA copies of the genes are synthesized as the first step leading to gene expression.
b. Messenger RNAs (mRNAs) carry copies of the genes that can be translated into proteins.
c. Other specialized RNAs, ribosomal RNA (rRNA), transfer RNAs (tRNAs), and small RNA molecules are not translated into protein but have central roles in gene expression and protein synthesis.
d. In eukaryotes, mRNAs are initially transcribed as heterogeneous nuclear RNA, which still contains intervening sequences of the gene and must undergo processing to attain the final mRNA structure.

II. Structure of Chromosomal DNA:

A. The information contained in the DNA is represented by the **sequence** of the bases of the polymer, the purines adenine (A) and guanine (G) and the pyrimidines cytosine (C) and thymine (T).

i. The deoxyribonucleotides in the DNA polymer are connected by phosphodiester bonds between the 5′-phosphate group attached to one deoxyribose sugar and the 3′-hydroxyl group of the next sugar.
ii. This sugar-phosphate backbone is located on the outside of the structure.

B. DNA is Double-Stranded:

i. The two strands run in reverse polarity or antiparallel to each other.
ii. The strands are held together by hydrogen bonding between the bases, with a purine always bonded with a pyrimidine in specific base pairs.

a. Base pairs have a preferred structural complementarity.
b. A pairs with T via two hydrogen bonds.
c. G pairs with C via three hydrogen bonds.

iii. The entire structure is twisted along the main axis in the form of a righthanded double helix.

a. In B DNA, the form that occurs most commonly under physiologic conditions, the helix has major and minor grooves, which provide access for protein binding to the DNA.
b. The double helix is also stabilized by hydrophobic base stacking interactions in the core.

iv. Cooperation between the many hydrogen bonds and base-stacking interactions makes DNA very stable to chemical treatments.

a. Increased heat, decreased salt concentration, or extremes of pH can force the DNA duplex to melt open (or “unzip”) by disrupting the hydrogen bonds between the strands in a process called denaturation.

b. The melting point (Tm) at which the DNA duplex is half-unzipped depends on length of the DNA and its percentage of G and C bases, which provide a stronger base-pair interaction than A and T pairs.
c. Nevertheless, the two strands of DNA must be separated by proteins under physiologic conditions for important processes like replication and transcription.

v. Deoxyribonucleases (DNAses) catalyze hydrolysis of phosphodiester bonds in the DNA backbone, leaving a 5′-phosphate and a 3′-hydroxyl on the ends.

a. Endonucleases cleave within the interior of a DNA strand, as in the case of restriction endonucleases, which cleave at sites having specific sequences.
b. Exonucleases cleave the last nucleotide from either the 3' or 5' ends of the DNA strand, depending on the enzyme's specificity.

C. Compacting of the DNA for storage in the limited space available in the cell's nucleus is accomplished by binding of proteins.

i. A family of small, basic proteins called histones is responsible for major interactions with DNA in the formation of nucleosomes.

a. Prokaryotic histones are of five types: H1, H2A, H2B, H3 and H4; vertebrates also have histone H5.
b. Binding of the positively charged histones to DNA neutralizes negative charges of the phosphate groups along the DNA backbone, which allows the DNA to bend much more easily than naked DNA.

ii. Two molecules each of the similarly sized histones H2A, H2B, H3, and H4 bind together in anoctamer that forms the core of a nucleosome.

a. DNA wraps around each nucleosome core 1.75 times, so that the nucleosomes form at uniform intervals along the DNA.
b. Approximately 146 base pairs of DNA are involved in forming each nucleosome, with about 30-base-pair linker regions between them.
c. Various types of histone H1 (or H5) bind loosely to the linker regions to help organize the nucleosomes into higher-order structures.

D. Tertiary and quaternary structures of DNA allow even further condensation of nucleosome-coated DNA into the highly compacted structure of the chromosome.

i. Nucleofilaments are organized by coiling the nucleosome-coated DNA into 30-nm fibers that also contain non-histone proteins and some RNA molecules.
ii. Packing of the nucleofilaments around scaffold proteins is the final level of condensation into chromosomes.
iii. Two copies of each chromosome are stored in the nucleus and take on a special structure during metaphase of mitosis prior to cell division.

a. The two chromosomes undergo further compaction into chromatids, the familiar dense structures visible when stained and viewed under the microscope.
b. Two sister chromatids are connected by a centromere composed of proteins bound to an A+T-rich region of the DNA.
c. The centromere forms the point of attachment for the mitotic spindle.

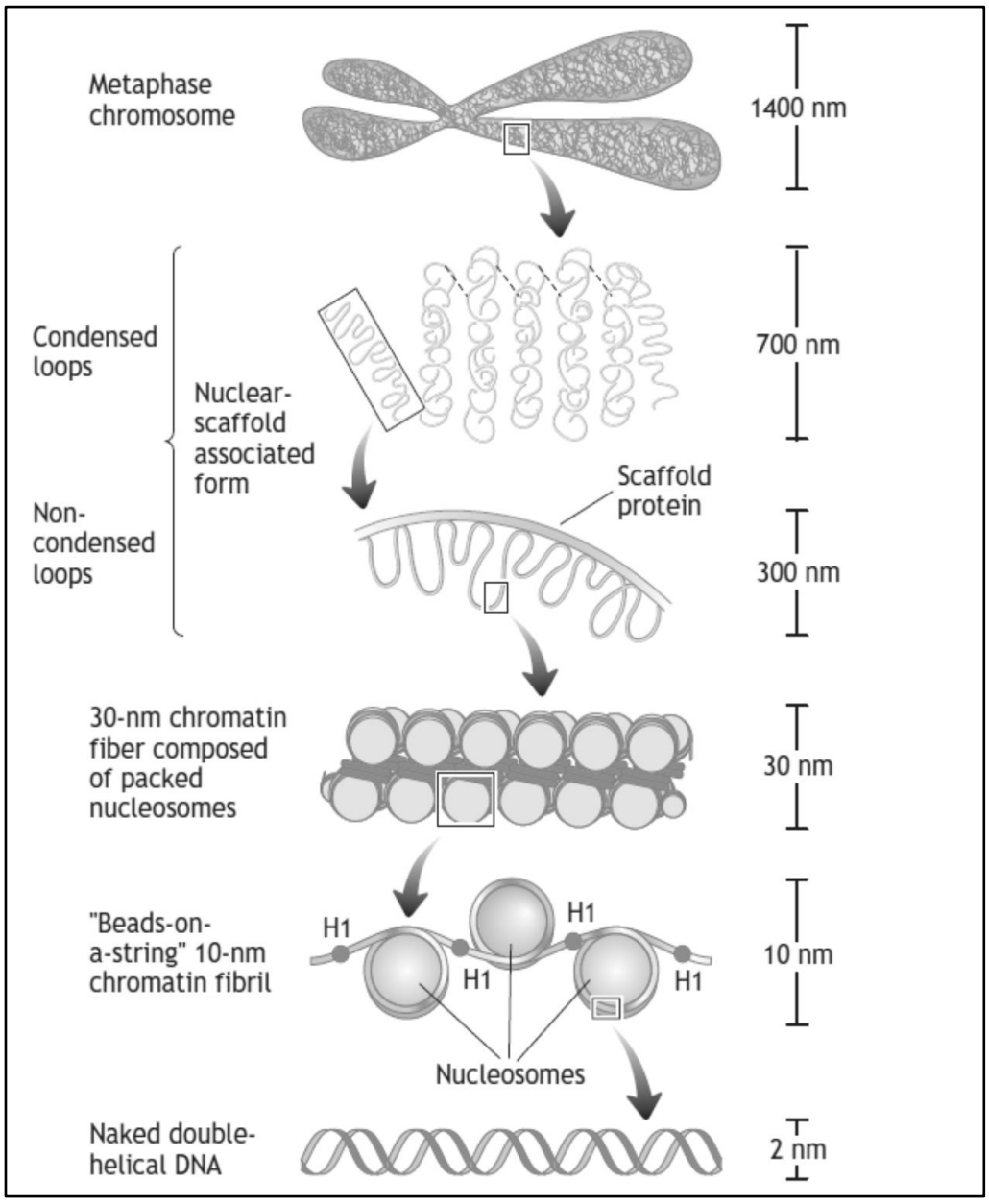


Figure 4.5: Compaction of DNA into a metaphase chromosome. Each phase of condensation or compaction reduces availability of DNA for replication or thanscription to the point where a metaphase chromosome is nearly inter for these processes. The overall, packaging reduces the length of the DNA 10^4-fold.

III. Replication:

A. In order that a complete complement of the genetic material may be inherited by daughter cells during cell division or by offspring from parents, the DNA must be copied with high fidelity by a process called DNA replication.

i. The DNA region to be replicated is copied by what is referred to as a semi-conservative mechanism.

a. The DNA region must be opened up from its double-stranded state to two halves of complementary, single-stranded DNA (ssDNA).
b. Each of the strands then serves as the template for synthesis of a new complementary, daughter strand.

ii. The two new daughter strands are formed along the template by base pairing with deoxynucleotide triphosphates (dNTPs) serving as the building blocks.

B. Prokaryotic DNA replication is accomplished by DNA polymerases, large multienzyme complexes that move out bidirectionally from the origin of replication.

i. DNA replication begins with protein binding to the origin of replication; a unique sequence in the bacterial chromosome, causing a short region of double- stranded DNA (dsDNA) to unwind.
ii. Single-stranded DNA binding proteins (SSBs) bind to this short region of ssDNA, which promotes further unwinding of a nearby A+T-rich region.
iii. DNA helicase, an enzyme that catalyzes DNA unwinding, can then bind to this region and begin working in cooperation with SSBs.

a. Helicase forces open dsDNA by breaking hydrogen bonds stabilizing the base pairs ahead of the moving replication fork.
b. SSBs then bind to the ssDNA to prevent reannealing to the double-stranded state.

iv. Once the replication fork is established, other proteins begin assembling the functional DNA replication complex.

a) DNA polymerases are able to copy the single-strand template DNA by operating only in a 5’ to 3’ direction.
b) This limitation presents a problem to copy DNA of the unzipped, antiparallel strands in opposing directions, both toward and away from the replication fork.
c) To solve this problem, different mechanisms are used to make dsDNA using the single-stranded templates on the forward (leading) strand and the retrograde (lagging) strand (Figure 4.5).

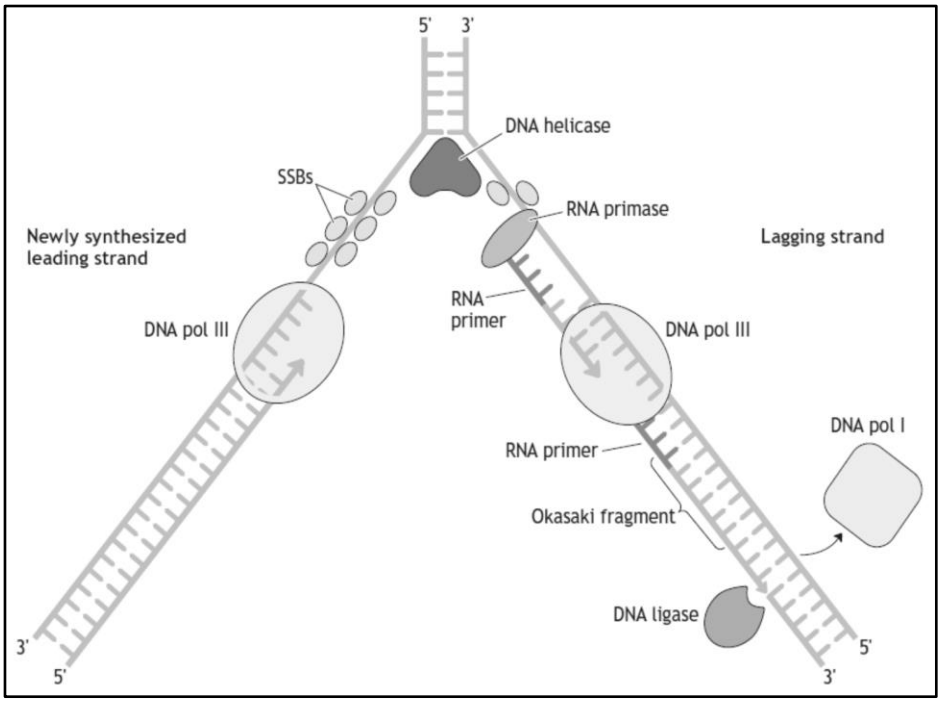


Figure 4.6: The prokarytic DNA replication fork. A schematic representation of semi-conservative replication of DNA by different mechanisms on the leading and lagging strands by DNA polymerase III (DNA pol III) is shown. Other enzymes and accessory proteins that particupate in initiation, elongation, and ligation phase of the process are indicated, with DNA pol I depicted as having just dissociated from completed Okasaki fragment. SSBs, single-stranded DNA binding proteins.

v. DNA polymerase III (DNA pol III), the main DNA polymerase of *Escherichia coli*, synthesizes DNA continuously on the leading strand and discontinuously on the lagging strand.

   a. DNA polymerases require that a primer with an available 3′-hydroxyl end be annealed to the template.
   b. The nascent or growing polynucleotide chain being made as complement to the leading strand continuously provides a 3′ end that is extended by DNA pol III.

vi. Replication on the lagging strand is discontinuous because polymerases can only copy the single stranded region available at the fork and only in the 5′ to 3′ direction.

   a. A short RNA primer is first synthesized nearest the 3′ end of the fork by primase, which is actually a DNA-directed RNA polymerase.
   b. DNA pol III binds to the primer-template end and extends the primer by adding deoxyribonucleotides during the elongation step.
   c. Short pieces of DNA called Okasaki fragments are made in this way and each fragment is completed when DNA pol III bumps up against the primer end of the previous fragment.

d. The RNA primers are excised and simultaneously replaced with DNA by DNA pol I, which also has 5'to 3' exonuclease activity.
e. DNA ligase then seals the remaining nick by catalyzing formation of a phosphodiester bond with ATP as energy donor.

III. Mutations and DNA Repair:

a. Mutations or heritable alterations in the DNA sequence that affect protein structure or gene expression can occur in many ways and may be passed to daughter cells during cell division.

i. Errors in DNA replication can produce a variety of mutations by failure of proofreading mechanisms.
ii. Point mutations or single base substitutions are classified as transitions or transversions.

- Transitions are defined as the substitution of one purine for another on the same strand (e.g., A to G or G to A); likewise for pyrimidine substitutions.
- Transversions are defined as the substitution of a purine for a pyrimidine or vice versa (e.g., A to C or T to G).

b. Chemical modification of DNA caused by environmental mutagens may lead to changes in the function or expression of genes.

i. Chemical reactions can modify DNA bases leading to altered base pairing in subsequent rounds of replication.

- Alkylating agents are compounds that are metabolized within cells to unstable species that react with sites on the DNA bases, which may alter their base-pairing properties and eventually cause mutations.
- Some compounds react with bases to produce adducts, which are covalently modified bases that are spontaneously ejected from the DNA. The abasic site formed as a result cannot base-pair properly upon replication.

ii. Intercalating agents are aromatic compounds that fit between the base pairs in the core of DNA structure and lead to insertions and deletions of one or more base pairs upon replication.

iii. Ultraviolet light causes neighboring thymine bases to form thymine dimmers that block replication and gene expression.

IV. RNA Structure:

A. All RNA molecules represent copies of genes on the cellular DNA, but there are some important differences in structure between DNA and RNA.

The features of RNA structure that distinguish it from DNA follow:

a. Presence of ribose as the sugar in the backbone of RNA rather than 2′-deoxyribose as in DNA.
b. Thymine (T) in DNA is replaced by uracil (U) in RNA.
c. RNA is a single-stranded version of one strand of the DNA sequence, at least as initially synthesized.
d. RNA can form complex, variable secondary structures by internal fold back and intramolecular base pairing between complementary regions of the molecule.

Most types of cellular RNA are involved in various steps in protein synthesis or gene expression.

B. The function of the ribosome, including its main catalytic activity, depends on several forms of ribosomal RNA (rRNA).

   a. Ribosomes are large nucleoprotein machines composed of large and small subunits that carry out protein synthesis.
   b. Prokaryotic ribosomes contain three rRNAs: 16S rRNA in the small (30S) subunit and 23S and 5SrRNA molecules in the large (50S) subunit.
   c. Eukaryotic ribosomes contain four rRNAs analogous to those in prokaryotes: the 18S rRNA of the small (40S) subunit and the 28S, 5.8S, and 5S of the large (60S) subunit.
   d. Cells have many ribosomes, so rRNAs comprise the majority (~80%) of cellular RNA.

C. mRNA represents an RNA copy of a gene, which directs synthesis of a specific protein by the ribosomes.

   a. Prokaryotic genes encode protein sequences directly with no intervening noncoding DNA, so that mRNA transcripts serve as direct templates for protein synthesis.
   b. In eukaryotes, the first step in mRNA synthesis is transcription of the template or "non-coding" strand of DNA into a large heterogeneous nuclear RNA (hnRNA), which undergoes processing to remove intervening, non-coding sequences (introns) and to add stabilizing structures.

D. tRNAs are small molecules that function as adaptors to convert or translate the nucleotide sequence information of mRNAs into the amino acid sequences of the proteins they encode.

   a. Many different forms of tRNA occur in cells, at least 1 for each of the 20 common amino acids.
   b. The tRNAs are 65–110 nucleotides long and their backbones fold back to allow for intramolecular hydrogen binding (base pairing or hybridization) to form a cloverleaf secondary structure.
   c. Base stacking effects and some unusual forms of hydrogen bonding between the bases cause tRNAs to take on a tertiary structure that is roughly L-shaped.

- The 3′ OH end of all tRNAs has the same sequence, 5′-CCA-3′, forming the acceptor stem to which a specific amino acid attaches.
- At the opposite side of the molecule, is the anticodon loop, containing the 3-base sequence or anticodon that base pairs with the codon, or amino acid-specifying unit, of the mRNA.
- Other loops such as the TC loop and DHU loop help the tRNA bind to various enzymes and to ribosomes.

d. The tRNAs undergo post-transcriptional modification to produce specialized bases, such as pseudouridine, dehydrouridine, and methylcytosine.

E. Small nuclear RNA (snRNA) molecules are components of splicesomes, which are complex nucleoprotein assemblies that process or splice hnRNAs to mRNAs.

V. Transcription:

I. Transcription is the process by which the template strand of DNA is copied into RNA for purposes of gene expression.
II. DNA-dependent RNA polymerase copies the sequence of the DNA template into a complementary RNA or transcript.

a. Like DNA polymerases, prokaryotic RNA polymerase (RNA pol) is a multiprotein complex that operates only in the 5′ to 3′ direction as it copies the template.

- The RNA pol **holoenzyme** has five subunits in its **$\alpha_2\beta\beta'\alpha$ complex.**
- The sigma factor, σ, can dissociate from the holoenzyme, leaving behind the **core enzyme,** which has the main catalytic activities.

b. The mechanism of transcription is identical for all forms of RNA and occurs in multiple steps.

c. To initiate transcription, the RNA pol holoenzyme binds to and slides (scans) along the DNA Searching for an appropriate promoter, a specific sequence element that indicates the 5′ end of a gene.

- The factor of the holoenzyme binds to the DNA sequence 5′-TATAAT-3′, called the TATA box, within the promoter region guiding the holoenzyme to the site.
- RNA pol holoenzyme unwinds 17 base pairs of DNA to form the preinitiation complex.
- RNA pol then forms the first phosphodiester bond between two base-paired ribonucleotides to initiate the new chain, in the absence of a primer.
- Once the first phosphodiester bond is formed, factor dissociates, which decreases the affinity of RNA pol for the promoter and allows the core enzyme to continue synthesis along the DNA.

D. Elongation of the transcript occurs by incorporation of ribonucleotides to create a copy or RNA Complement of the DNA template.

- The RNA pol holoenzyme, the unwound portion of the template and the nascent RNA chain form the transcription bubble, which moves along the DNA during transcription (Figure 11–3).
- Ribonucleotides are added to the nascent chain according to base-pairing rules, with C hydrogen bonding with G as usual and U pairing with A of the DNA and a pairing with T of the DNA.
- Topoisomerases prevent supercoiling ahead of and behind the moving bubble.
- RNA pol does not have nuclease activity, so it is not capable of proofreading and is more error-prone than DNA polymerase.

Termination of transcription occurs when RNA pol traverses a termination signal, and this process may require the cooperation of ρ (rho) factor.

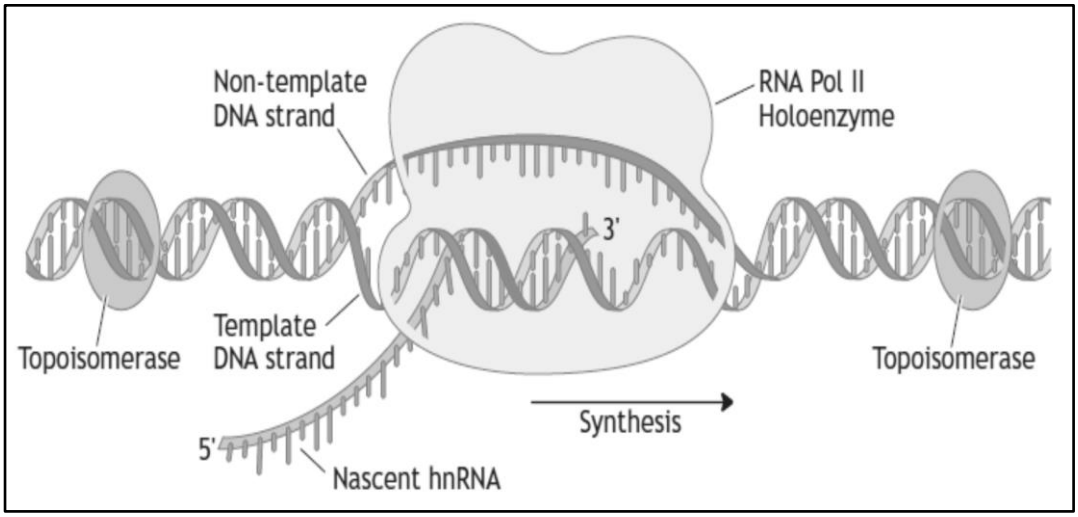


Figure 4.7: The prokarytic RNA transcription bubble. RNA pol II, RNA polymerase II: hnRNA, heterogeneous nuclear RNA.

C. Eukaryotic transcription is more complex than in prokaryotes, mainly in terms of the nature of the RNA polymerases, the assembly of the pre-initiation complex, and the need for processing eukaryotic RNAs.

I. Three DNA-dependent RNA polymerases operate in the transcription of eukaryotic genes.

- **a.** RNA pol I transcribe the 28S, 18S, and 5.8S rRNA genes, an activity that is localized to the nucleolus, a region of high nucleoprotein density in the cell's nucleus.
- **b.** RNA pol II is responsible for transcription of snRNA genes and of structural genes encoding mRNAs leading to protein synthesis.
- **c.** RNA pol III transcribes the tRNA genes and the 5S rRNA gene.

II. General Transcription Factors (GTFs) that bind to eukaryotic promoters are functionally analogous to σ factor in prokaryotes.

a. **TATA binding protein (TBP)** recognizes the **TATA box** element of the promoter on type II genes (those transcribed by RNA pol II), binds to it in a sequence-specific manner, and recruits other GTFs to form a complex.
b. RNA pol II is then attracted to the complex to form the pre-initiation complex.
c. Besides TBP, the GTFs and more specific transcription factors that regulate transcription of the many type II genes differ depending on the gene.

4.11 Chapter at a Glance:

Term	Description
IMP	Inosine monophosphate
PRPP	5'-phosphoribosyl-1-pyrophosphate
THF	Tetrahydrofolate
NSAIDs	Non-Steroidal Analgesic and anti-inflammatory agents
dNTPs	Deoxynucleotide triphosphates
DNA	Deoxyribonucleic acid

4.12 Exercises:

4.12.1 Multiple choice Questions:

1. A nucleoside consists of

(a) Nitrogenous base
(b) Purine or pyrimidine base + sugar
(c) Purine or pyrimidine base + phosphorous
(d) Purine + pyrimidine base + sugar +phosphorous

2. In the biosynthesis of c-DNA, the joining enzyme ligase requires

(a) GTP
(b) ATP
(c) CTP
(d) UTP

3. Template-directed DNA synthesis occurs in all the following except

(a) The replication fork
(b) Polymerase chain reaction
(c) Growth of RNA tumor viruses
(d) Expression of oncogenes

4. In RNA molecule

(a) Guanine content equals cytosine
(b) Adenine content equals uracil
(c) Adenine content equals guanine
(d) Guanine content does not necessarily equal its cytosine content.

5. In contrast to eukaryotic mRNA, prokaryotic mRNA

(a) Can be polycistronic
(b) Is synthesized with introns
(c) Can only be monocistronic
(d) Has a poly a tail

6. The size of small stable RNA ranges from

(a) 0–40 nucleotides
(b) 40–80 nucleotides
(c) 90–300 nucleotides
(d) More than 320 nucleotides

7. Thymine is present in

(a) tRNA
(b) Ribosomal RNA
(c) Mammalian mRNA
(d) Prokaryotic mRNA

8. Double helical structure model of the DNA was proposed by

(a) Pauling and Corey
(b) Peter Mitchell
(c) Watson and Crick
(d) King and Wooten

9. DNA does not contain

(a) Thymine
(b) Adenine
(c) Uracil
(d) Deoxyribose

10. The sugar moiety present in DNA is

(a) Deoxyribose
(b) Ribose
(c) Lyxose
(d) Ribulose

4.12.2 Short Answer Questions:

1. Explain Gout.
2. Define mutarotation.
3. What is phosphorolysis?
4. What do you mean by mutation in genes?
5. Write a note on genetic transformation of enzymes.

4.12.3 Long Answer Questions:

1. Give an account of Biosynthesis of purine and pyrimidine nucleotides
2. Give the Structure of DNA and RNA and their functions
3. Write a note on DNA replication
4. Give an account on Transcription or RNA synthesis
5. Write a note on Translation or Protein synthesis and inhibitors

Answer key MCQs:

(1) – (b), (2) – (b), (3) – (c), (4) – (d), (5) – (b), (6) – (c), (7) – (c), (8) – (c), (9) – (c), (10) – (c)

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Chapter 5

Enzymes

5.1 Introduction:

Enzymes are biological molecules (typically proteins) that significantly speed up the rate of virtually all of the chemical reactions that take place within cells. Thousands of chemical reactions proceed very rapidly at any given instant within all living cells of an organism. Virtually all of these reactions are mediated by remarkable molecular devices called enzymes. That is, the enzymes are central to every biochemical reaction and are called the catalysts of biological systems (biocatalysts).

The term 'enzyme' was coined by Kuhne (1878) for catalytically active substances previously called ferments. Enzymes were actually found out by Buchner (1897) with the accidental discovery that fermentation of sugar is not only caused by living yeast cells but also yeast extract. The extract obviously possessed biocatalysts required for the process. Buchner (1903) also isolated the first enzyme. He was awarded Nobel Prize in the same year, 1903.

Enzymes are mainly functional inside the living cells. As found out by Buchner they can be extracted from the cells and made to catalyse reactions outside the living cells. In nature some enzymes are secreted by living cells and made to perform extracellular catalysis. Enzymes functional outside the living cells are called exo-enzymes, e.g., enzymes present in digestive juices, lysozyme of tears. Enzymes functional inside living cells are known as endoenzymes, e.g., enzymes of Krebs cycle (inside mitochondria), enzymes of glycolysis (inside cytoplasm).

5.2 Importance of Enzyme:

5.2.1 Biological Importance of Enzymes:

a. Thousands of chemical reactions are taking place in the body of a living organism. All of them are mediated by enzymes,
b. Enzyme mediated reactions do not require harsh treatment,
c. They are pH specific so that reactions requiring different pH operate in different parts of the body,
d. Enzymes play an important role in Metabolism, Diagnosis, and Therapeutics.
e. Enzymes are specialized catalysts that operate at biological temperatures,
f. As they operate under favourable conditions, enzymes force the organisms to live under favourable environment,
g. Enzymes are highly regulated. Their formation is controlled by separate genes. Activation and repression of genes allow certain enzymes to be functional or non-functional in cells.

5.2.2 Examples of Specific Enzymes:

There are thousands of enzymes in the human body; here are just a few examples:

a. Lipases: a group of enzymes that help digest fats in the gut.
b. Amylase: helps change starches into sugars. Amylase is found in saliva.
c. Maltase: also found in saliva; breaks the sugar maltose into glucose. Maltose is found in foods such as potatoes, pasta, and beer.
d. Trypsin: found in the small intestine, breaks proteins down into amino acids.
e. Lactase: also found in the small intestine, breaks lactose, the sugar in milk, into glucose and galactose.
f. Acetylcholinesterase: breaks down the neurotransmitter acetylcholine in nerves and muscles.
g. Helicase: unravels DNA.
h. DNA polymerase: synthesize DNA from deoxyribonucleotides.

- **Nomenclature:**

Enzymes are generally named according to the reaction they catalyze or by suffixing “ase” after the name of substrate. The International Union of Biochemistry and Molecular Biology developed a nomenclature for enzymes. Each enzyme is described by a sequence of four numbers preceded by "EC". EC denotes Enzyme Commission and the number of enzyme is called EC numbers.

5.2.3 Classification of Enzyme:

A. Enzymes are broadly of two types:

- **Intracellular or Endoenzymes:**
  - They are functional within cells where they are synthesized.
- **Extracellular or Exoenzymes:**
  - These enzymes are active outside the cells.
  - **Example:** Digestive enzymes like Pepsin, Trypsin, and Amylase.

B. Classification of Enzymes by IUB System:

Enzymes are classified by complex system, suggested by commission on enzymes of International Union of Biochemistry (IUB). Based on their action they are divided into 6 major classes. Each enzyme is assigned a 4 Digit code number.

a. Oxido-Reductases:
- Enzymes in this class are involved in Oxidation-Reduction reactions.
- **Example:** Alcohol Dehydrogenase.

b. Transferases:

- Enzymes that catalyze transfer of Functional groups are called as Transferases.

- **Example:** Phosphorylases

c. Hydrolases:

- These are enzymes that bring about hydrolysis of various compounds.
- **Example:** Lipase

d. Lyases:

- Enzymes specialized in addition or removal of water.
- **Example:** Aldolase

e. Isomerases:

- Enzymes involved in all isomerization reactions.
- **Example:** Phosphotriose Isomerase.

f. Ligases:

- Enzymes catalyzing synthetic reactions where two molecules are joined together and ATP are used.
- **Example:** Succinate thiokinase

Enzyme class	Reaction type	Description
EC1 Oxidoreductases	$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Redox reactions: Two classes viz. oxidase and reductase.
EC2 Transferases	$A\text{-}B + C \longrightarrow A + B\text{-}C$	Transfer or exchange of certain groups among some substrates.
EC 3 Hydrolases	$A\text{-}B + H_2O \longrightarrow A\text{-}H + B\text{-}OH$	Facilitates hydrolysis of substrates.
EC 4 Lyases	$A\text{-}B \rightleftharpoons A + B$ (reverse reaction: synthase)	Removal of a group from the substrate to leave a double bond reaction or catalyze its reverse reaction.
EC 5 Isomerases	$A\text{-}B\text{-}C \rightleftharpoons A\text{-}C\text{-}B$	The facilitation of isoisomers, geometric isomers or optical isomers.
EC 6 Ligases	$A + B + ATP \longrightarrow A\text{-}B + ADP + P_i$	Facilitates the synthesis of two molecular substrates into one molecular compound with the release energy.

Enzymes can also be classified by the kind of chemical reaction catalyzed.

a. Addition or Removal of Water:

- Hydrolases - these include esterases, carbohydrases, nucleases, deaminases, amidases, and proteases
- Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase

b. Transfer of Electrons:

- Oxidases
- Dehydrogenases

c. Transfer of a Radical:

- Transglycosidases - of monosaccharides
- Transphosphorylases and phosphomutases - of a phosphate group
- Transaminases - of amino group
- Transmethylases - of a methyl group
- Transacetylases - of an acetyl group

d. Splitting or Forming a C-C Bond:

- Desmolases

e. Changing Geometry or Structure of a Molecule:

- Isomerases

f. Joining Two Molecules through Hydrolysis of Pyrophosphate Bond in ATP or Other Tri-Phosphate:

- **Ligases:**

Moreover, on the basis of the molecular composition, enzymes can be divided into pure enzymes and binding enzymes. Enzymes containing only protein are called pure enzymes. Binding enzymes are composed of proteins and cofactors. Only when the two components are combined, can the enzyme have catalytic activity.

5.2.4 Specificity of Enzymes:

One of the properties of enzymes that makes them as important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction.

Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

A. Absolute Specificity: the enzyme will catalyze only one reaction.

- Urease acts only on urea to produce ammonia and CO_2.

$H_2N\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

B. Group Specificity: the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

- Lactate dehydrogenase (LDH) catalyses the interconversion of pyruvic acid and lactic acid and also number of other structurally related compounds.

  i. $H_3C\text{-}CO\text{-}COOH + NADH + H^+ \leftrightarrow H_3C\text{-}CHOHCOOH + NAD^+$

- Pepsin splits a peptide bond in which carboxyl group is donated by dicarboxylic acid and amino group is donated by aromatic amino acid.
- Trypsin splits peptide bond in which carboxyl group is contributed by either lysine or arginine.
- Chymotrypsin splits peptide bond in which carboxyl group is from an aromatic amino acid. Thrombin splits peptide bond in which carboxyl group is donated by arginine and amino group is from glycine.

One enzyme acts on more than one substrate and conversely a substrate catalysed by more than one enzyme. E.g. sucrase acts on both sucrose and raffinose. Raffinose is also catalysed by melibiase.

C. Linkage Specificity: the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

D. Stereochemical Specificity: the enzyme will act on a particular steric or optical isomer. Some enzymes exhibit specificity towards cis and Trans forms. E.g. fumarase acts on fumaric acid (Trans form) and not on maleic acid which is cis isomer of fumaric acid.

E.g. arginase acts only on L-arginine and not on its D-isomer (D-arginine). Similarly D-amino oxidase oxidises the D-amino acids and not L-amino acids. Some enzymes interconvert the two optical isomers of a compound. E.g. alanine racemase catalyses the interconversion between L- and D-alanine.

L-alanine ↔ D-alanine

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes.

For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

5.3 Energy Changes:

The way enzymes work can also be shown by looking at the energy changes during a chemical reaction.

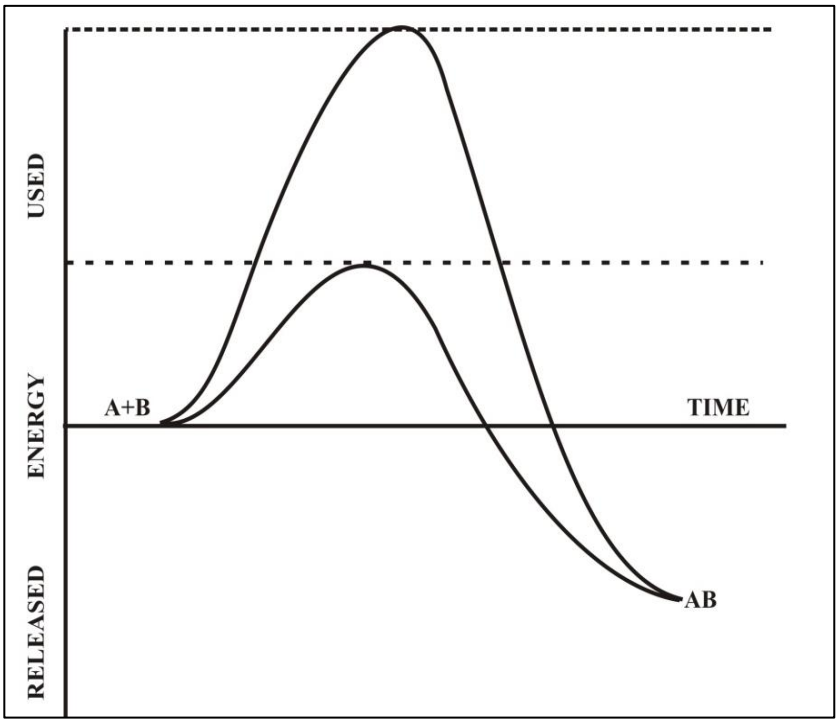
In a reaction where the product has a lower energy than the substrate, the substrate naturally turns into product (i.e. the equilibrium lies in the direction of the product).

Before it can change into product, the substrate must overcome an "energy barrier" called the activation energy.

The larger the activation energy is, the slower the reaction will be. This is because only a few substrate molecules will have sufficient energy to overcome the activation energy barrier.

Imagine pushing boulders over a hump before they can roll downhill, and you have the idea. Most biological reactions have large activation energies, so they without enzymes they happen far too slowly to be useful.

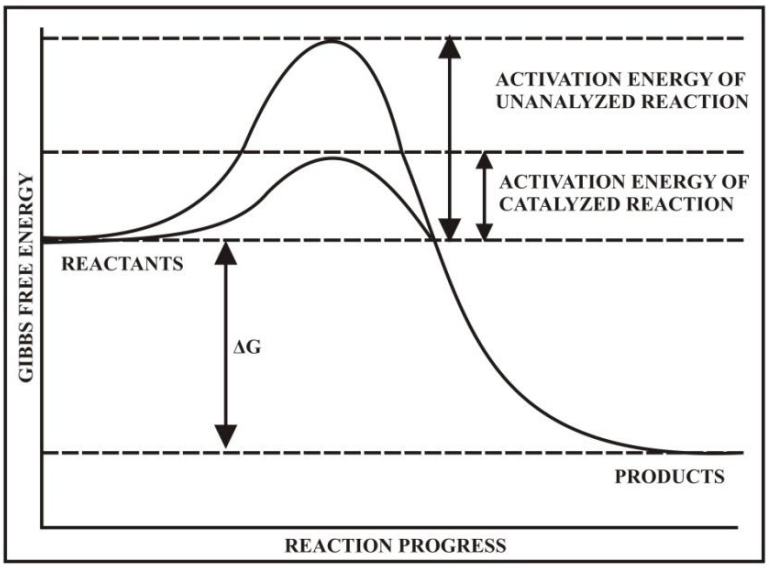
Enzymes reduce the activation energy of a reaction so that the kinetic energy of most molecules exceeds the activation energy required and so they can react.



For example, for the catalase reaction ($2H_2O_2 \rightarrow 2H_2O + O_2$) the activation energy is 86 kJ mol -1 with no catalyst, 62 kJ mol -1 with an inorganic catalyst, and just 1 kJ mol -1 with the enzyme catalase.

5.4 Enzymes and Activation Energy:

Enzymes perform the critical task of lowering a reaction's activation energy—that is, the amount of energy that must be put in for the reaction to begin. Enzymes work by binding to reactant molecules and holding them in such a way that the chemical bond-breaking and bond-forming processes take place more readily.



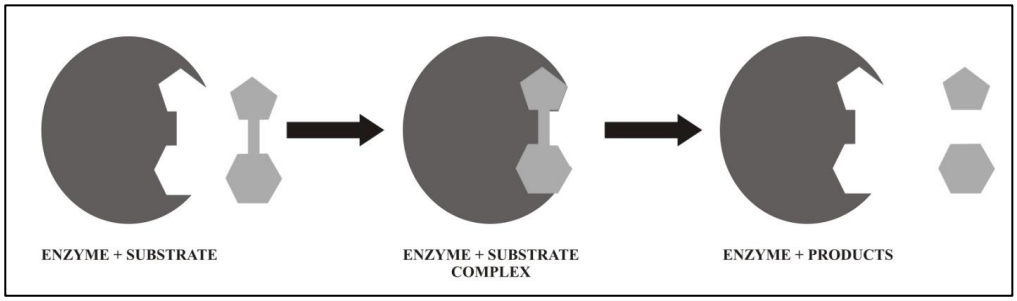
Reaction coordinate diagram showing the course of a reaction with and without a catalyst. With the catalyst, the activation energy is lower than without. However, the catalyst does not change the ΔG for the reaction. To clarify one important point, enzymes don't change a reaction's ΔG value. That is, they don't change whether a reaction is energy-releasing or energy-absorbing overall. That's because enzymes don't affect the free energy of the reactants or products.

Instead, enzymes lower the energy of the **transition state,** an unstable state that products must pass through in order to become reactants. The transition state is at the top of the energy "hill" in the diagram above.

5.5 The Mechanism of Enzyme Action:

5.5.1 Enzyme Action:

a. Enzymes differ widely in structure and specificity, but a general theory that accounts for their catalytic behavior is widely accepted.
b. The enzyme and its substrates interact only over a small region of the surface of the enzyme, called the active site.
c. When the substrate binds to the active site via some combination of intermolecular forces, an enzyme-substrate (ES) complex is formed.
d. Once the complex forms, the conversion of the substrate (S) to product (P) takes place:



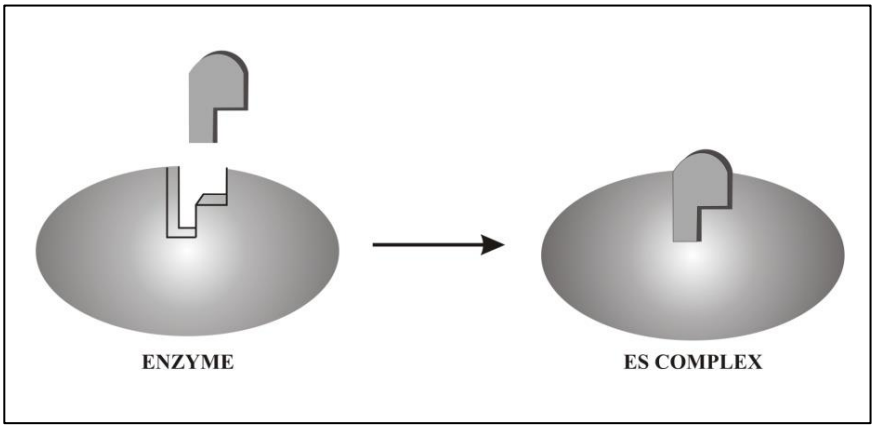
e. The chemical transformation of the substrate occurs at the active site, aided by functional groups on the enzyme that participate in the making and breaking of chemical bonds.

After the conversion is complete, the product is released from the active site, leaving the enzyme free to react with another substrate molecule.

5.5.2 Lock-and-Key Theory:

The "lock and key" model was first proposed in 1894. In this model, an enzyme's active site is a specific shape, and only the substrate will fit into it, like a lock and key.

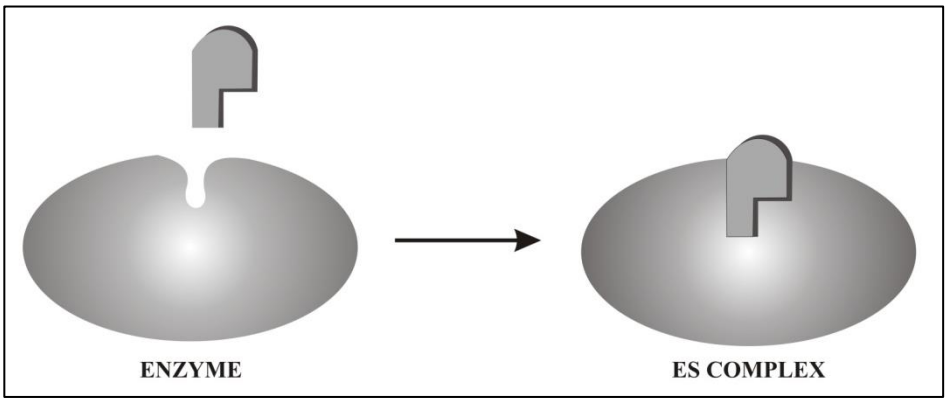
The lock-and-key theory explains the high specificity of enzyme activity. Enzyme surfaces accommodate substrates having specific shapes and sizes, so only specific substances “fit” in an active site to form an ES complex. A limitation of this theory is that it requires enzymes conformations to be rigid. Research suggests that instead enzymes are at least somewhat flexible.



5.5.3 Induced-Fit Theory:

A modification of the lock-and-key theory called the induced-fit theory proposes that enzymes have flexible conformations that may adapt to incoming substrates.

The active site adopts a shape that is complementary to the substrate only after the substrate is bound.



5.5.4 Enzyme Kinetics:

- The mechanism of enzymatic reactions has been studied since a long time by analyzing the protein composition of enzymes and their steric structures. At the beginning of the 20th century, the German scientists Leonor Michaelis and Maud Menten, who were studying an enzyme called invertase, later proposed a method for describing enzyme kinetics based on the following experimental facts.

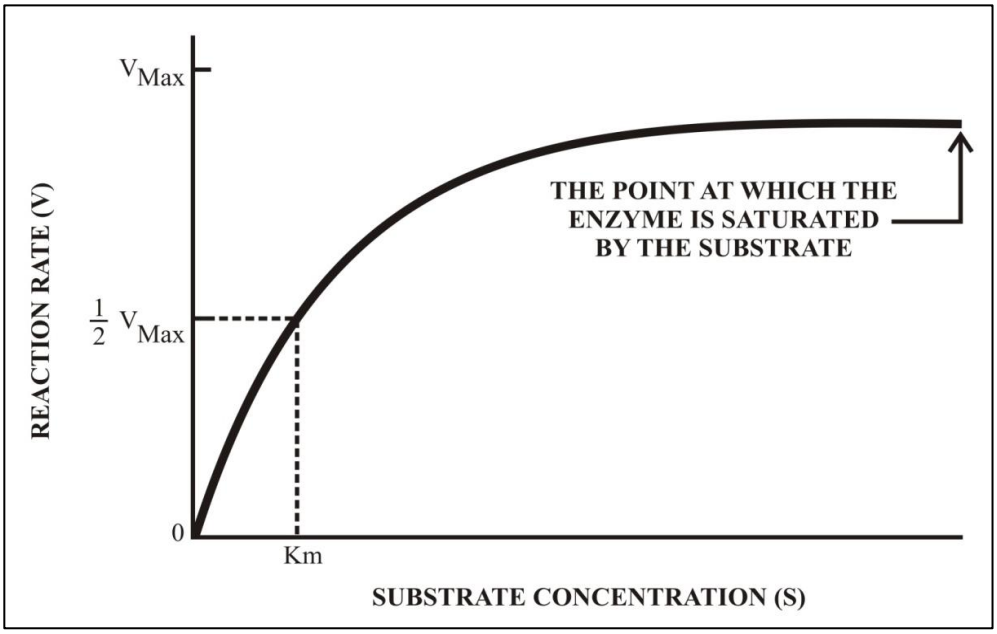


Figure 5.1: The Relationship between the Enzymatic Reaction Rate and Substrate Concentration

- In an enzymatic reaction, the initial reaction rate V is increased by increasing the concentration [S] of the substrate S. However, in cases where the amount of enzyme added to the reaction system is fixed, no matter how much you increase [S], V cannot increase over a certain limit.

This extrapolated value is called Vmax. In other words, V is saturated with regard to [S]. The phenomenon of saturation is characteristic of catalytic reactions and is explained as the binding of a catalyst and substrate. The phenomenon of saturation is observed because even if there is an abundance of substrate molecules, the number of substrate binding sites available for the catalyst is limited.

- This is shown by a mathematical formula, and study of the rate of an enzymatic reaction is called kinetics. A simple enzymatic reaction is considered below. Invertase is an enzyme that hydrolyzes sucrose to produce glucose and fructose. Although a large quantity of water is involved in this reaction, it does not have an effect on the reaction rate, and hence, only sucrose (represented by [S]) is considered to be the substrate. The enzyme molecule is represented by E.

Although there are two types of products formed, they are both represented by P. The concentration of the reaction products is considered in a range that does not affect the reaction rate.

Important assumptions made here include the assumption that the enzyme and substrate bind reversibly and that a portion of the bonded substrate and enzyme will yield products through the primary reaction.

$$\mathbf{E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{K_2} E + P}$$

At this point, the following general equations are established.

$$v = \frac{v_{max}}{1 + k_m/[S]} \quad \text{And} \quad k_m = \frac{k_{-1} + k_2}{k_1}$$

These are called the Michaelis–Menten equations. Here V_{max} refers to the maximum initial reaction rate, and K_m refers to the Michaelis constant. Km represents [S] that gives an initial reaction rate of ½ V_{max}. This equation is essentially a hyperbolic function demonstrating the phenomenon of saturation. The equation is derived on the basis of certain assumptions that in reality do not always hold true for all enzymatic reactions. However, in practice, the equation holds true for many enzymatic reactions, and Km is used as an index showing affinity between the enzyme and substrate (smaller the K_m, greater the affinity). One

application of this formula is the classification of inhibitors of enzymatic reactions. Although inhibitors are classified according to their effect on K_m and V_{max}, among those, only competitive inhibitors will be discussed here. When a substance with a structure similar to that of a substrate binds to the substrate binding site of the enzyme but does not participate in an enzymatic reaction, it interferes with the original substrate reaction and is called a competitive inhibitor.

For example, succinate dehydrogenase, an enzyme in the citric acid cycle, is inhibited by malonic acid (HOOC-CH_2-COOH), a substance analogous to succinic acid (HOO-CH_2-CH_2-COOH). Thus, the observed K_m increases, but V_{max} does not change, in the presence of the inhibitor.

In addition, allosteric regulation is another factor that exerts an effect on enzymatic activity. Allosteric regulation is commonly observed in enzymes composed of multimers. During this regulation, the steric structure of the constituent components change cooperatively when a regulator molecule binds to a site (the allosteric site) other than the active center.

This causes the multimer to alternately switch between the high and low activity states depending on the large changes in the conformational state of the enzyme due to the allosteric regulator.

5.5.5 Derivation of the Michaelis−Menten Equation:

In 1925, George Briggs and John Haldane derived the currently used Michaelis–Menten equation.

In the simple enzymatic reaction mentioned in this textbook, dependence of the initial reaction rate on the concentration of the substrate is considered. If the total concentration of the enzyme is set as E_0, then

$E_0 = [E] + [ES]$ ---------- (1)

At stable conditions assumed, no change is observed in the concentration of the intermediate ES,

$$\frac{d[ES]}{dt} = k_1[S][E] - (k_{-1} - k_2)[ES] = 0$$

Which can be rewritten as follows:

$[S][E] - K_m [ES] = 0$ ---------- (2)

Because [S] >> [ES], the concentration of the separated substrate is considered the overall concentration of the substrate.

If [E] is removed from (1) and (2), then

$$[ES] = \frac{E_0[S]}{[S] + Km} \text{---------- (3)}$$

By substituting (3) in the equation for the initial reaction rate V, the result obtained is as follows

$$V = k_2[ES] = \frac{k_2 E_0}{1 + k_m/[S]} = \frac{V_{max}}{1 + k_m/[S]}$$

This gives $V_{max} = k_2E_0$. Note that the enzyme E and the substrate S are in rapid equilibrium, and if k_1, $k_{-1} >> k_2$, then km can give a dissociation constant for the enzyme and substrate of

$$k = \frac{k_{-1}}{k1}$$

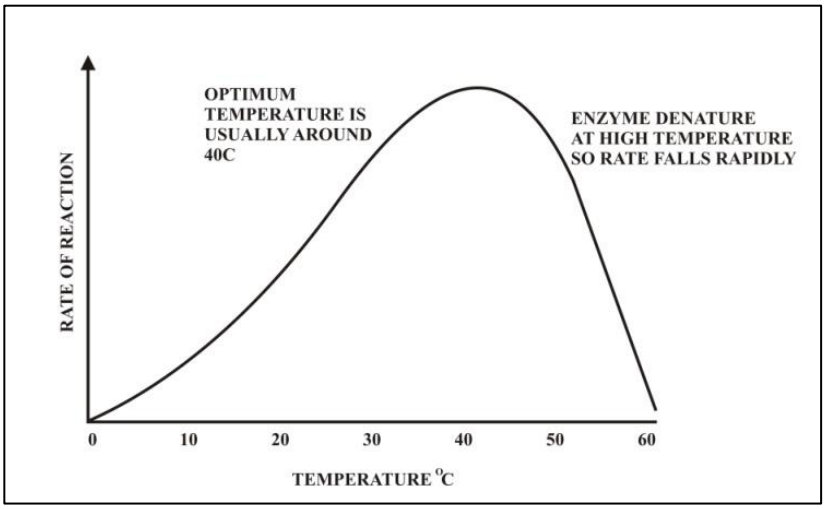
5.5.6 Factors that Affect the Rate of Enzyme Reactions:

a. Temperature:

Enzymes have an optimum temperature at which they work fastest. For mammalian enzymes this is about 40°C, but there are enzymes that work best at very different temperatures, e.g. enzymes from the arctic snow flea work at -10°C, and enzymes from thermophilic bacteria work at 90°C.

Up to the optimum temperature the rate increases geometrically with temperature (i.e. it's a curve, not a straight line).

The rate increases because the enzyme and substrate molecules both have more kinetic energy and so collide more often, and also because more molecules have sufficient energy to overcome the activation energy.

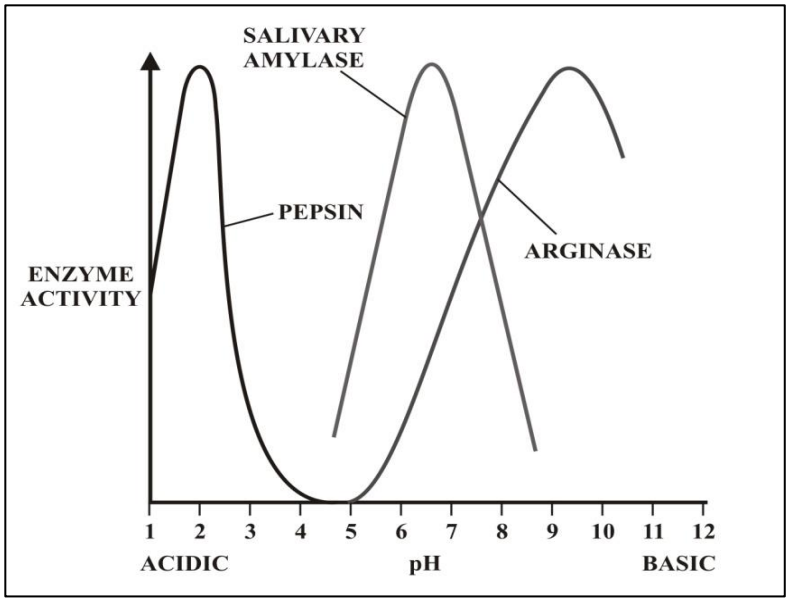


$$\left(Q10 = \frac{rate\ of\ temp\ (t + 10)\ °C}{rate\ of\ temp\ t\ °C}\right)$$

Above the optimum temperature the rate decreases as more of the enzyme molecules denature. The thermal energy breaks the hydrogen bonds holding the secondary and tertiary structure of the enzyme together, so the enzyme loses its shape and becomes a random coil - and the substrate can no longer fit into the active site. This is irreversible. Remember that only the hydrogen bonds are broken at normal temperatures; to break the primary structure (the peptide bonds) you need to boil in strong acid for several hours – or use a protease enzyme! The increase in rate with temperature can be quantified as the Q_{10}, which is the relative increase for a 10°C rise in temperature. Q_{10} is usually around 2 for enzyme-catalyzed reactions (*i.e. the rate doubles every 10°C*) and usually less than 2 for non-enzyme reactions. The rate is not zero at 0°C, so enzymes still work in the fridge (and food still goes off), but they work slowly. Enzymes can even work in ice, though the rate is extremely slow due to the very slow diffusion of enzyme and substrate molecules through the ice lattice.

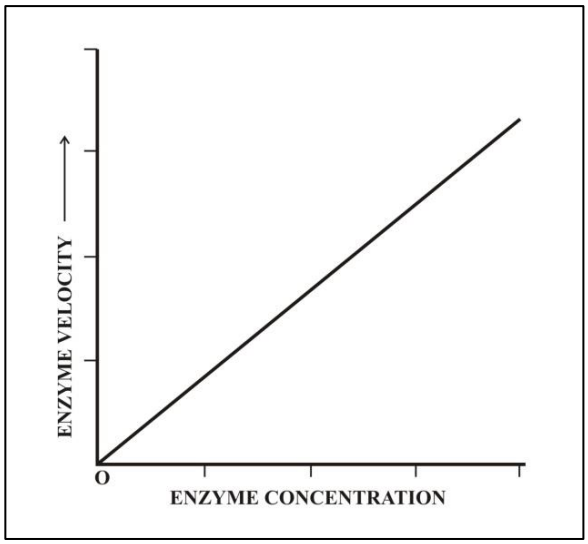
b. pH:

Enzymes have an optimum pH at which they work fastest. For most enzymes this is about pH 7-8 (normal body pH), but a few enzymes can work at extreme pH, such as gastric protease (pepsin) in our stomach, which has an optimum of pH 1. The pH affects the charge of the amino acids at the active site, so the properties of the active site change and the substrate can no longer bind. For example a carboxyl acid R groups will be uncharged a low pH (COOH), but charged at high pH (COO-).



c. Enzyme Concentration:

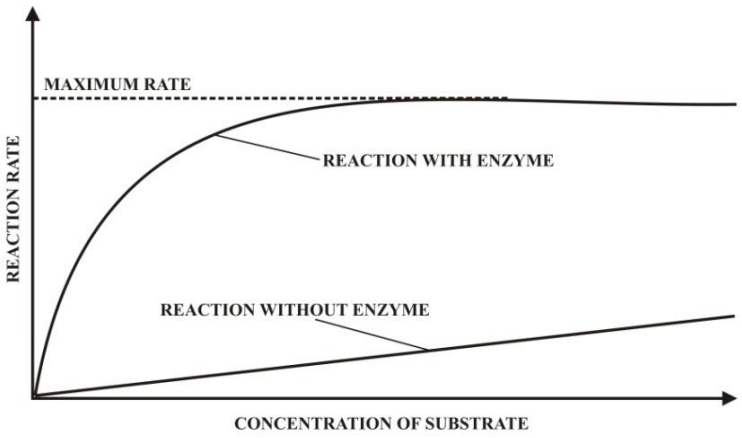
As the enzyme concentration increases the rate of the reaction also increases, because there are more enzyme molecules (and so more active sites), available to catalyse the reaction therefore more enzyme-substrate complexes form. In cells, the substrate is always in excess, so the graph does not level out. In the lab, these conditions need not apply and a plateau can be reached.



5.5.7 Effect of Enzyme Concentration on Enzyme Activity:

a. Substrate Concentration:

The rate of an enzyme-catalyzed reaction is also affected by substrate concentration. As the substrate concentration increases, the rate increases because more substrate molecules can collide with active sites, so more enzyme-substrate complexes form.



At higher concentrations the enzyme molecules become saturated with substrate, and there are few free active sites, so adding more substrate doesn't make much difference (though it will increase the rate of E-S collisions).

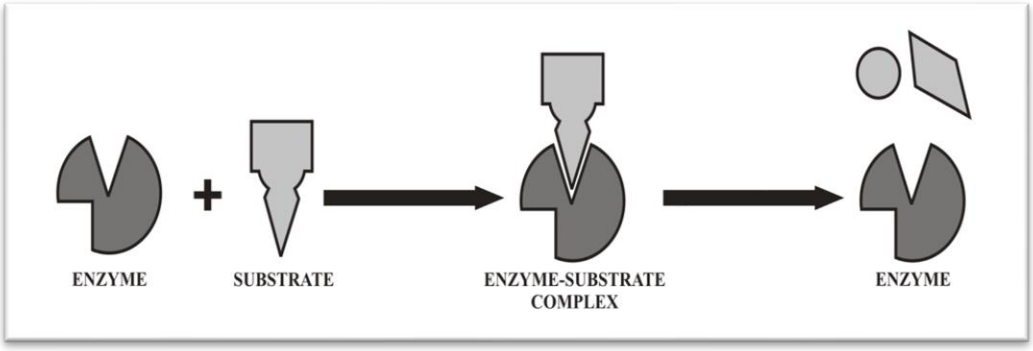
The maximum rate at infinite substrate concentration is called v_{max}, and the substrate concentration that gives a rate of half v_{max} is called K_M. These quantities are useful for characterizing an enzyme. A good enzyme has a high v_{max} and a low K_M.

b. Enzyme Inhibition:

Enzyme activity can be inhibited by certain chemical substances called inhibitors. Enzyme inhibitors have provided information about substrate specificity of enzymes, the nature of functional groups at active site and the mechanisms of the catalytic activity. Some drugs considered to be useful in medicine function by inhibiting certain enzymes, for example, the inhibition of bacterial enzymes which affects the bacterial metabolism and, therefore, their growth and multiplication.

c. Normal Enzyme Reaction:

In a normal reaction, a substrate binds to an enzyme (via the active site) to form an enzyme-substrate complex. The shape and properties of the substrate and active site are complementary, resulting in enzyme-substrate specificity. When binding occurs, the active site undergoes a conformational change to optimally interact with the substrate (induced fit). This conformational change destabilises chemical bonds within the substrate, lowering the activation energy. As a consequence of enzyme interaction, the substrate is converted into product at an accelerated rate.



Enzyme inhibitors are of two types: Irreversible and reversible.

a. Irreversible Inhibitors:

These inhibitors bind covalently to the enzymes causing the permanent loss of their catalytic activity by altering or destroying a functional group on the enzyme molecules. Arsenic, lead, mercury and various insecticides are all irreversible inhibitors. Even at low concentration of these the enzymes are precipitated.

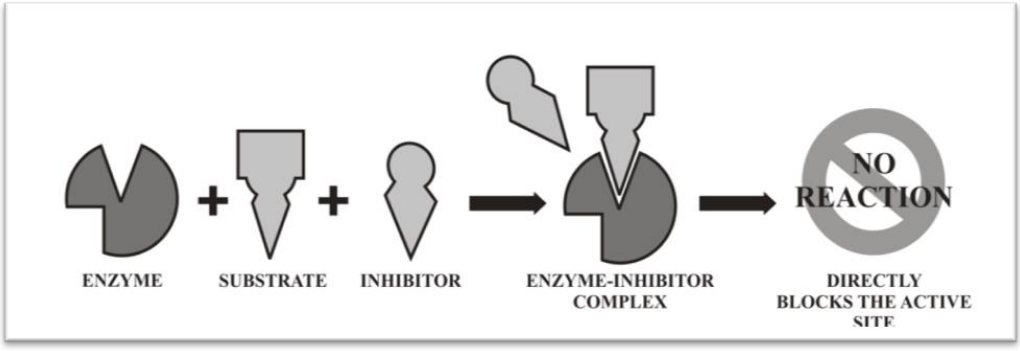
b. Reversible Inhibitors:

These inhibitors bind non-covalently to enzymes and their effects can be reversed. Important information on the structure of the active sites of various enzymes has been obtained with the help of reversible inhibitors.

The two main types of reversible inhibitors are competitive and noncompetitive inhibitors.

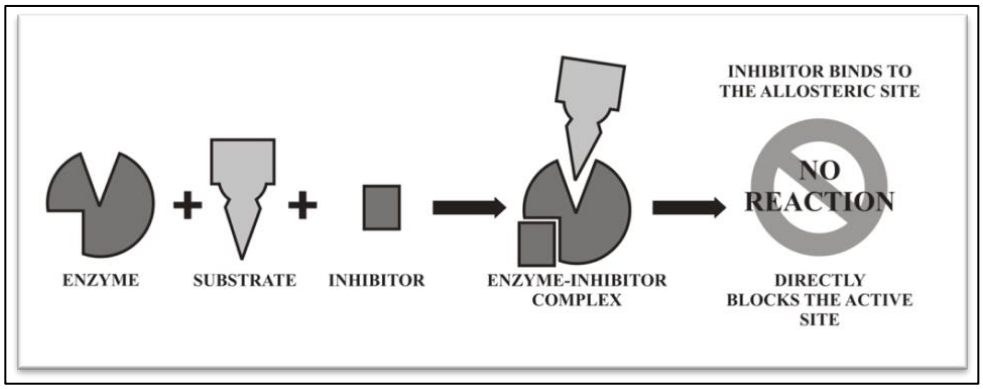
- **Competitive Inhibitors:**

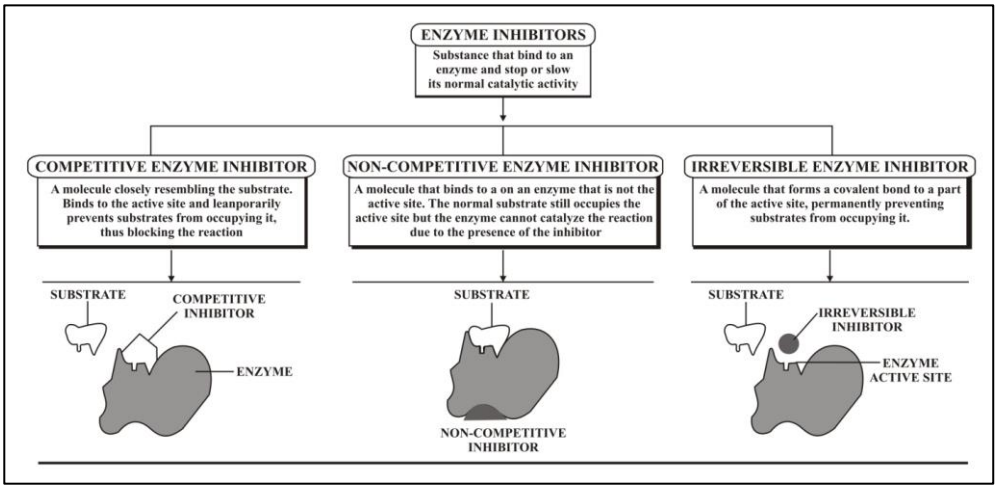
Competitive inhibitors compete with the real substrate for binding to the active site of the enzyme because they resemble the substrate molecules. The catalytic ability of the enzyme is not affected by this binding but the ability of the substrate to bind to the enzyme is reduced. However, these inhibitors cannot be transformed into products. Competitive inhibition is reversed simply by increasing the substrate concentration. For example, succinate dehydrogenase, which catalyses the removal of 2 hydrogen atoms from succinate, is inhibited by malonate which resembles succinate structurally. However, malonate is not dehydrogenated by the enzyme succinate dehydrogenase. It simply occupies the active site. The inhibition is reversed by increasing the succinate concentration.



- **Non-Competitive Inhibitors:**

These inhibitors bind at a site on the enzyme other than the catalytic sites, i.e. substrate binding site, altering the conformation of the enzyme molecule and leading to the distortion and inactivation of the catalytic site. Noncompetitive inhibitors bind reversibly to both the free enzyme and the ES complex to form the inactive complexes. Addition of more substrate will not restore the previous rate of reaction in contrast to competitive inhibitors. Important noncompetitive inhibitors are the naturally occurring metabolic intermediates for example L-thonine dehydratase is inhibited by L-isoleucine. Competitive and noncompetitive inhibitors are not necessarily harmful. Both are used extensively by cells for metabolic regulation.



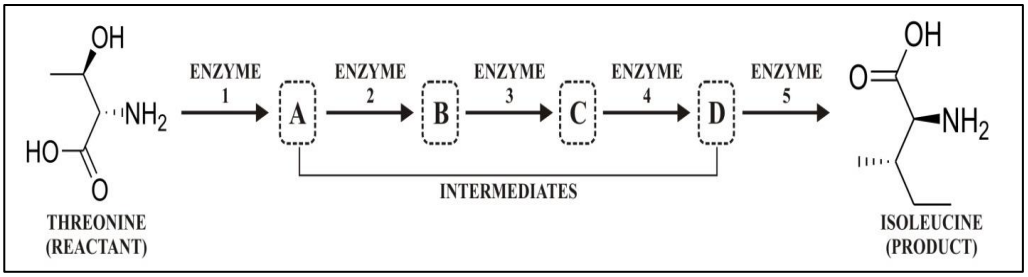


5.6 Allosteric Regulation:

- Compounds that alter enzymes by changing the 3D conformation of the enzyme are called modulators.
- They may increase the activity (activators) or decrease the activity (inhibitors). (Noncompetitive inhibitors are examples of this activity.)
- Enzymes with quaternary structures with binding sites for modulators are called allosteric enzymes.
- These variable-rate enzymes are often located at key control points in cell processes.
- Feedback inhibition occurs when the end product of a sequence of enzyme-catalyzed reactions inhibits an earlier step in the process. This allows the concentration of the product to be maintained within very narrow limits.

The synthesis of isoleucine from threonine is an example of allosteric regulation.

a. Threonine deaminase, which acts in the first step of the conversion pathway, is inhibited by the isoleucine product.
b. When isoleucine builds up, it binds to the allosteric site on threonine deaminase, changing its conformation so that threonine binds poorly. This slows the reaction down so that the isoleucine concentration starts to fall.
c. When the isoleucine concentration gets too low, the enzyme becomes more active again, and more isoleucine is synthesized.



5.7 Enzymes Inhibitors as Pharmaceutical Agents:

Cyclooxygenase (COX) is the enzymes are the major targets responsible for nonsteroidal anti-inflammatory drugs. The drugs belonging to this class are called as COX inhibitors are currently used in the treatment of patients with fever, cardiovascular diseases, joint pain, etc.

Among these drugs are both irreversible and reversible inhibitors that retard production of prostaglandins that control many aspects of inflammation, smooth muscle contraction, and blood clotting. But there are several other groups of drugs that are by nature of inhibitors of some enzymes; the following groups of enzyme inhibitors are developed now by pharmaceutical companies and have very essential remedial significances.

The new agents in the pharmaceutical field are inhibitors of angiotensin-converting enzyme (ACE). ACE is the main catalyst for the conversion of inactive decapeptide angiotensin I into angiotensin II by the removal of a dipeptide from the C-terminus of angiotensin I. Angiotensin II is a potent vasoconstrictor.

Inhibition of ACE results in the reduction of angiotensin I concentration and in the relaxation of smooth muscles of vessels. Inhibitors of ACE are widely used as drugs for treatment of arterial hypertension.

Proton pump inhibitors (PPIs). Is an enzyme that is located in the plasma membrane of the parietal cells of stomach mucosa? It is a P-type ATPase that provides proton secretion from parietal cells in gastric cavity against the electrochemical gradient using energy of adenosine triphosphate (ATP) cleavage. PPIs are groups of compounds from substituted benzopyridines that in acid medium of stomach are converted into active sulfonamides interacting with cysteine residues of pump.

Therefore, PPIs are acid-activated prodrugs that are converted into drugs inside the organisms. These drugs are successfully used for treatment of gastritis, gastric and duodenal ulcer, and gastroesophageal reflux disease from 80^{th} years of the twentieth century. Antibiotic penicillin covalently modifies the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria.

Statins represent a group of compounds that are analogs of mevalonic acid. They are inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, an enzyme participating in cholesterol synthesis. Statins are used as drugs preventing or slowing the development of atherosclerosis. Because of the existence of some adverse effects, statins may be recommended for patients that cannot achieve a decrease of cholesterol level in the blood through diet and changes in lifestyle.

Methotrexate is competitive inhibitor of a coenzyme for the enzyme dihydrofolate reductase, a structural analog of tetrahydrofolate, which catalyzes necessarily step in the biosynthesis of purines and pyrimidines. Methotrexate is very potent analog which binds to this enzyme approximately 1000-fold more tightly than the substrate and inhibits nucleotide base synthesis. It is used for cancer therapy.

The Nucleoside reverse transcriptase inhibitors and protease inhibitors are now recommended for treatment of patients with this decease are breakthrough in treatment of patients with acquired immune deficiency syndrome (AIDS) that is provoked by human immunodeficiency virus (HIV) was achieved recently using two different types of enzyme inhibitors... These inhibitors affect also some other viral infections and demonstrated anticancer activity. Presented here list of enzyme inhibitors that are used in therapy of numerous deceases that is far from being complete. Sciences around the world are involved in a search of new inhibitors of known enzymes that have therapeutic significance. An example of this complex research is a work devoted to design, synthesis, and study of new inhibitors of carbonic anhydrase, an enzyme that is involved in the development of such symptoms and deceases as edema, glaucoma, obesity, cancer, epilepsy, and osteoporosis.

5.8 Therapeutic and Diagnostic Applications of Enzymes and Isoenzymes:

The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance as Diagnostic Enzymes. This is because the presence of these enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood.

Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Because of their role in maintaining life processes, the assay and pharmacological regulation of enzymes have become key elements in clinical diagnosis and therapeutics.

Developments of medical applications for enzymes have been at least as extensive as those for industrial applications, reflecting the magnitude of the potential rewards: for example, pancreatic enzymes have been in use since the nineteenth century for the treatment of digestive disorders.

The variety of enzymes and their potential therapeutic applications are considerable.

S. No.	Enzymes	Therapeutic use	Basic	Additional information
1	Prolactazyme	Lactose intolerance	Prolactazyme is a proezmye that produces lactase in stomach.	About 75% of the world's pipulation is intolerant to lactose in adulthood. It occurs due to lack of lactase in digestive system.
2	Beta-Lactamase	Penicillin allergy	Penicillin is coverted to penicilloate	Learn more about penicillin allergy here
3	Aglucerase	Gaucher's Disease type I	Enzyme replacement therapy	This disease is characterized by the lack of enzyme glucocerebrocidase
4	Asparaginase	Acute childhood leukemia	Decreased level of serum asparagine and inhibition of	Tumor cells connot synthesize L- asparagine due to lack of aspartateammonia ligase

S. No.	Enzymes	Therapeutic use	Basic	Additional information
			aspargine dependent multiplication of tumor cells.	
5	Collagenase	Skin ulcers	Causes collagen hydrolysis	Break up and remove dead skin and tissue
6	DNAse	Cystic Fibrosis (CF)	DNAse hydrolyses extracellular DNA responsible for Cystic fibrosis	DNA present in the mucous, which arises from dead WBCs and bacterial cell. serves to cross link the mocous, changing of from a fluid gel to a semisolid
7	Lysozyme	Antibiotic Therapy	Causes Bacterial cell wall hydrolysis	
8	Ribonuclease	Antiviral Therapy	Causes RNA hydrolysis	
9	Typsin	Inflammation	Causes protein hydrolysis	
10	Uricase	Cout	Converts Urate to allantiori	
11	Enzyme inhibitor	To increase the efficacy of drugs	Against resistant bacteria	Example: Beta Lactamase inhibitor

5.9 Isoenzymes and their Diagnostic Importance:

Isoenzymes (or isozymes) are a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies. Isozymes are usually distinguished by their electrophoretic mobilities. They are usually separated from each other in the diagnostic laboratory via electrophoresis.

All living systems apparently require multiple molecular forms of certain enzymes in order to maximize biological capacity. Isoenzymes (also called isozymes) are alternative forms of the same enzyme activity that exist in different proportions in different tissues.

Isoenzymes differ in amino acid composition and sequence and multimeric quaternary structure; mostly, but not always, they have similar (conserved) structures.

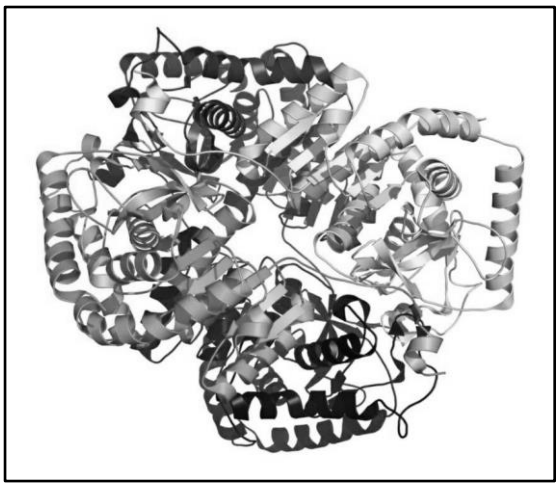
5.10 Lactate Dehydrogenase (LDH):

The measurement of LDH is especially diagnostic for myocardial infarction because this enzyme exists in five closely related, but slightly different forms (isozymes).

Lactate ←*LDH*→ Pyruvate

5.10.1 LDH Is a Tetrameric Enzyme with 2 Types of Subunit "H" and "M":

a. M (for muscle) : basic
b. H (for heart) : acidic



Lactate dehydrogenase structure (LDH)

5.10.2 Isoenzymes of LDH:

The 5 types and their normal distribution and levels in non-disease/injury are listed below.

a. LDH1 (H4) : Heart and RBC
b. LDH2 (H3M) : Heart and RBC
c. LDH3 (H2M2) : Brain and kidney
d. LDH4 (HM3) : Liver and skeletal muscle
e. LDH5 (M4) : Liver and skeletal muscle

LDH1 has high Km (low affinity) and LDH5 has low Km (high affinity) for pyruvate.

Following a myocardial infarction, the serum levels of LDH rise within 24-48 hours reaching a peak by 2–3 days and return to normal in 5-10 days.

Especially diagnostic is a comparison of the LDH-1/LDH-2 ratio. Normally, this ratio is less than 1.

A reversal of this ratio is referred to as a "flipped LDH". Following an acute myocardial infarction, the flipped LDH ratio will appear in 12–24 hours and is definitely present by 48 hours in over 80% of patients.

Also important is the fact that persons suffering chest pain due to angina only will not likely have altered LDH levels.

5.10.3 Diagnositc Importance of LDH:

a. Normal: LDH2 > LDH1
b. Myocardial infarction (within 12-24 hours): LDH1>>LDH2 (flipped LDH pattern)
c. Liver diseases: increased LDH5 in serum
d. Increased LDH suggests other following diseases:

- Hemolytic anemia
- Hypotension
- Infectious mononucleosis
- Intestinal ischemia and infarction
- Muscle injury
- Muscular dystrophy
- Pancreatitis
- Lung tissue death
- Stroke
- Ischemic cardiomyopathy

5.11 Creatine Phosphokinase (CPK):

CPK is found primarily in heart and skeletal muscle as well as the brain. Therefore, measurement of serum CPK levels is a good diagnostic for injury to these tissues. The levels of CPK will rise within 6 hours of injury and peak by around 18 hours. If the injury is not persistent the level of CK returns to normal within 2–3 days.

Phosphocreatine ←*CPK*→ Creatine

I. CPK is a dimeric enzyme consisting of 2 subunits:

a. M : for muscle
b. B : for brain

II. Isoenzymes of CPK:

a. CPK1 (BB) : Brain
b. CPK2 (MB) : Heart
c. CPK3 (MM) : Skeletal muscles

III. Diagnositic Importance of CPK:

a. Normal: low CPK2 (<2%) in serum
b. MI (within 6-18 hrs): increased CPK2 (20%) in serum
c. Increased CPK-1 Suggests:

- Brain cancer
- Brain injury
- Pulmonary infarction
- Seizure

IV. Increased CPK-2 Suggests Other Diseases Like:

- Electrical injuries
- Heart injury
- Myocarditis

V. Increased CPK-3 Suggests:

- Crush injuries
- Rhabdomyolysis
- Muscular dystrophy
- Myositis
- Recent seizures

5.12 Alkaline Phosphatase (ALP):

The enzyme is a monomer and the isoenzymes are due to the difference in the carbohydrate content:

a. Alpha 1-ALP
b. Alpha 2-Heat labile ALP
c. Alpha 2-Heat stable ALP
d. Pre Beta-ALP
e. Gamma-ALP etc.

5.12.1 Diagnostic Importance of ALP:

i. Increased Alpha2-Healt labile ALP:

- **Liver diseases :** Biliary obstruction, hepatitis

ii. **Increased Pre Beta-ALP:**

- **Bone diseases:** Paget's disease, Osteoblastic bone tumors, Osteomalacia, Rickets, Skeletal disease

iii. **Increase in ALP also suggests other Diseases Like:**

- Pregnancy
- Healing bone fracture
- Anemia
- Leukemia
- Thyroid gland inflammation
- Hyperparathyroidism
- Chronic alcohol ingestion

iv. Decreased ALP Suggests:

- Malnutrition

5.13 Coenzymes –Structure and Biochemical Functions:

5.13.1 Coenzymes and Cofactors:

A large number of enzymes require an additional non - protein component to carry out its catalytic functions called as cofactors. A non-protein compound or metal or coenzyme ion that is covalently bound to the enzyme protein is called prosthetic group.

5.13.2 Cofactors-Two Types:

a. Inorganic ions such as $Fe^{2}+$, $Mg^{2}+$, $Mn^{2}+$, $Zn^{2}+$
b. A complex organic molecule called coenzyme. Some enzymes require both a coenzyme and one or more metal ions for their activity. Coenzymes function as transient carriers of specific functional groups.

5.14 Function of Coenzymes:

An enzyme without a coenzyme is called an apoenzyme. Without coenzymes or cofactors, enzymes cannot catalyze reactions effectively. In fact, the enzyme may not function at all. If reactions cannot occur at the normal catalyzed rate, then an organism will have difficulty sustaining life.

When an enzyme gains a coenzyme, it then becomes a holoenzyme, or active enzyme. Active enzymes change substrates into the products an organism needs to carry out essential functions, whether chemical or physiological. Coenzymes, like enzymes, can be reused and recycled without changing reaction rate or effectiveness.

They attach to a portion of the active site on an enzyme, which enables the catalyzed reaction to occur. When an enzyme is denatured by extreme temperature or pH, the coenzyme can no longer attach to the active site.

5.14.1 Types of Enzymes:

Cofactors are molecules that attach to an enzyme during chemical reactions. In general, all compounds that help enzymes are called cofactors. However, cofactors can be broken down into three subgroups based on chemical makeup and function:

a. Coenzymes:

These are reusable non-protein molecules that contain carbon (organic). They bind loosely to an enzyme at the active site to help catalyze reactions. Most are vitamins, vitamin derivatives, or form from nucleotides.

- **There are a Few Related Terms also Related to Coenzymes:**

a. **Apoenzym**e is the name given to an inactive enzyme that lacks its coenzymes or cofactors.
b. **Holoenzym**e is the term used to describe an enzyme that is complete with its coenzymes and cofactors.
c. **Holoprotein** is the word used for a protein with a prosthetic group or cofactor.

A coenzyme binds to a protein molecule (the apoenzyme) to form an active enzyme (the holoenzyme).

b. Cofactors:

Unlike coenzymes, true cofactors are reusable non-protein molecules that do not contain carbon (inorganic). Usually, cofactors are metal ions such as iron, zinc, cobalt, and copper that loosely binds to an enzyme's active site.

They must also be supplemented in the diet as most organisms do not naturally synthesize metal ions.

c. Prosthetic Groups:

These can be organic vitamins, sugars, lipids, or inorganic metal ions. However, unlike coenzymes or cofactors, these groups bind very tightly or covalently to an enzyme to aid in catalyzing reactions. These groups are often used in cellular respiration and photosynthesis.

d. Vitamins as Coenzymes:

Coenzymes molecules are often vitamins or are made from vitamins. Many coenzymes contain the nucleotide adenosine as part of their structures, such as ATP, coenzyme A and NAD^+. Coenzymes 1.Thiamine 2.Riboflavin 3.Niacin 4.Pantothenic acid 5.Pyridoxine 6.Biotin 7.Cobalamin 8.Folic acid and 9.Lipoic acid

5.15 Chapter at a Glance:

Term	Description
IUB	International Union of Biochemistry
LDH	Lactate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
COX	Cyclooxygenase
ACE	Angiotensin-converting enzyme
PPI	Proton pump inhibitors

5.16 Exercises:

5.16.1 Multiple Choice Questions:

1. Holoenzyme is

(a) Functional unit (b) Apo enzyme
(c) Coenzyme (d) All of these

2. Example of an extracellular enzyme is

(a) Lactate dehydrogenase (b) Cytochrome oxidase
(c) Pancreatic lipase (d) Hexokinase

3. Enzymes, which are produced in inactive form in the living cells, are called

(a) Papain (b) Lysozymes
(c) Apoenzymes (d) Proenzymes

4. An example of lyases is

(a) Glutamine synthetase (b) Fumarase
(c) Cholinesterase (d) Amylase

5. A sigmoidal plot of substrate concentration ([S]) verses reaction velocity (V) may indicate

(a) Michaelis-Menten kinetics (b) Co-operative binding
(c) Competitive inhibition (d) Non-competitive inhibition

6. An inducer is absent in the type of enzyme:

(a) Allosteric enzyme (b) Constitutive enzyme
(c) Co-operative enzyme (d) Isoenzymic enzyme

7. Fischer's 'lock and key' model of the enzyme action implies that

(a) The active site is complementary in shape to that of substance only after interaction.
(b) The active site is complementary in shape to that of substance
(c) Substrates change conformation prior to active site interaction
(d) The active site is flexible and adjusts to substrate

8. A demonstrable inducer is absent in

(a) Allosteric enzyme (b) Constitutive enzyme
(c) Inhibited enzyme (d) Co-operative enzyme

9. An enzyme which uses hydrogen acceptor as substrate is

(a) Xanthine oxidase (b) Aldehyde oxidase
(c) Catalase (d) Tryptophan oxygenase

10. in reversible non-competitive enzyme activity inhibition

(a) Vmax is increased (b) Km is increased
(c) Km is decreased (d) Concentration of active enzyme is reduced

5.16.2 Short Answer Questions:

1. Write a note on ETC.
2. Write a note on co-enzyme.
3. What do you mean by allosteric enzyme inhibition?
4. Give Line Weaver Burke plot for enzymes.
5. Give the classification of enzymes.

5.16.3 Long Answer Questions:

1. Write a note on enzyme inhibition and MM equation and plot of enzyme kinetics.
2. What are isoenzymes and explain its diagnostic applications.
3. Give an account for Enzyme inhibitors with examples.
4. Give the detailed account for Coenzymes –Structure and biochemical functions.
5. Write a note on enzyme induction and repression.

Answer key MCQs:

(1) – (d), (2) – (c), (3) – (c), (4) – (b), (5) – (b), (6) – (b), (7) – (b), (8) – (c), (9) – (d), (10) – (b).

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Chapter 6

Lipid Metabolism

6.1 Introduction:

Lipids are the group of heterogeneous compounds which are water-insoluble (Lipophilic) organic molecules extracted from tissues by nonpolar solvents. Due to their insolubility in aqueous solutions, in body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles or on albumin.

Lipids are a most important resource of energy for the body, and they provide the hydrophobic barrier. Lipids serve additional functions in the body, for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis.

6.1.1 Classification of Lipids:

a. Simple Lipids:

Fatty acid esters with various alcohols.

- **Fats:** Fatty acids esters with glycerol. Oils are liquid state fats.
- **Waxes:** Fatty acids esters with large molecular weight monohydric alcohols.

b. Complex Lipids:

Fatty acids esters with additional groups with alcohol and a fatty acid.

- **Phospholipids:** Lipids which contains phosphoric acid residue in addition to fatty acids and an alcohol. These molecules frequently have nitrogen bases and other substituents, e.g., in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
- **Glycolipids (Glycosphingolipids):** Lipids containing a fatty acid, sphingosine, and carbohydrate.
- **Other Complex Lipids:** Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

c. Precursor and Derived Lipids:

These comprise fatty acids, glycerol, steroids, other alcohol alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins and hormones.

Fatty acids exist mainly as esters in natural fats and oils but do occur in the unesterified form as free fatty acids, a transport form found in the plasma.

The natural fats are generally straight-chain derivatives containing an even number of carbon atoms.

The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds).

Saturated Fatty Acids may base on acetic acid (CH_3COOH) as the first member of the series in which **-CH_2-** is progressively added between the terminals **-CH_3-** and **-COOH-** groups.

Unsaturated Fatty Acids contain one or more double bonds and it may be further subdivided as follows:

a. Monounsaturated (monoethenoid, monoenoic) acids, containing one double bond.
b. Polyunsaturated (polyethenoid, polyenoic) acids, containing two or more double bonds.
c. **Eicosanoids:** These compounds, derived from eicosa- (20-carbon) polyenoic fatty acids, comprise the prostanoids, leukotrienes (LTs), and lipoxins (LXs). Prostanoids include prostaglandins (PGs), prostacyclins (PGIs), and thromboxanes (TXs).

6.1.2 Saturated Fatty Acids:

Common Name	**Number of C Atoms**	
Acetic	2	Major end product of carbohydrate fermentation by rumen organisms
Propionic	3	An end product of carbohydrate fermentation by rumen organisms
Butyric	4	In certain fats in small amounts (especially butter). End product of carbohydrate fermentation by rumen organisms.
Valeric	5	
Caporoic	6	
Lauric	12	Spermaceti, cinnamon, palm kernel, coconut oils, laurels, butter
Myrictric	14	Nutmeg, palm kernel, coconut oils, laurels, myrtles, butter
Palmitic	16	Common in all animal and plant fats
Stearic	18	

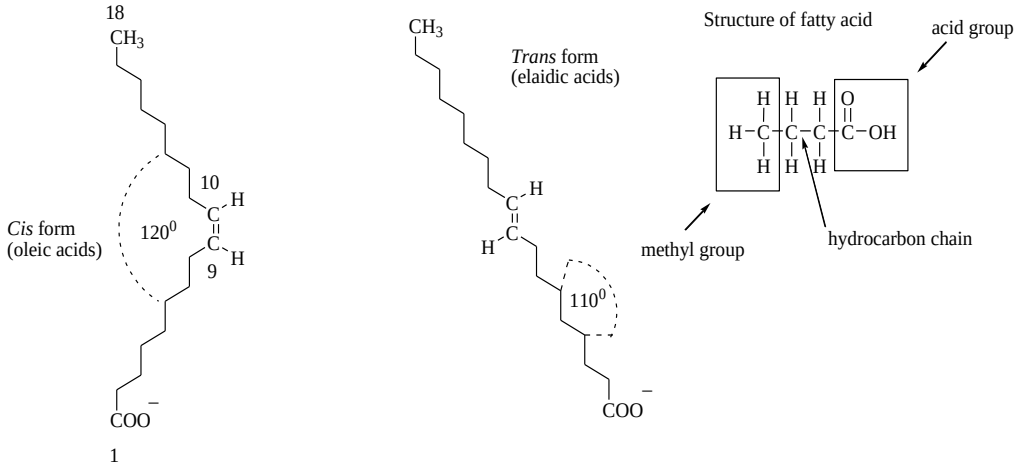
6.1.3 Unsaturated Fatty Acids:

Number of C Atoms and number and position of Double Bounds	Common Name	Occurrence
Monoenoic acid (One double bond)		
16:1'9	Palmitoleic	In nearly all fats.
18:1;9	Oleic	Possibly the most common fatty acid in natural fats.
18:1;9	Elaidic	Hydrogenated and ruminant fats.
Dienoic acid (two double bonds)		
18:2;9,12	Linoleic	Corn, peanut, cottonseed, soybean, and many plant oils.
Trienoic acids (three double bonds)		
18:3;6.9.12	y-Linoienic	Some plants eg. oil of evening primrose, borage oil minor fatty acid in animal
18:3;9,12,15	ά-Linoienic	Frequently found with linoleic acid but particularly in linseed oil
Tetraenoic acid (four double bonds)		
20:4;5,8,11,14	Arachidonic	Found in animal fats and peanut oil important component of phospholipids in animal

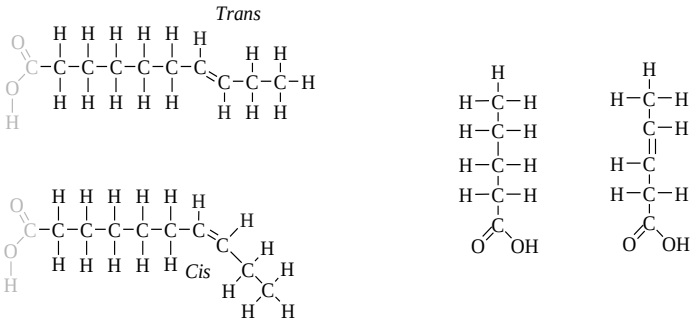
6.1.4 Chemistry of Fatty Acids:

a. There is a zigzag patteren of carbon chains of saturated fatty acids when extended, as at low temperatures. In the reverse condition of higher temperatures, some bonds rotate, causing chain shortening,
b. Depending on the orientation of atoms or groups around the axes of double bonds that do not permit rotation, a geometric isomerism occurs in unsaturated fatty acids. If the acyl chains are on the same side of the bond, it is *cis*-, as in oleic acid; if on opposite sides, it is *trans*-, as in elaidic acid, the *trans*- isomer of oleic acid.

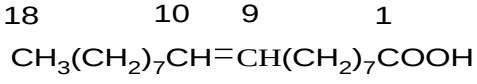
c. Unsaturated long-chain fatty acids which are naturally occurring are nearly the entire cis configuration, the molecules being "bent" 120 degrees at the double bond. Thus, oleic acid has an L shape, whereas elaidic acid remains "straight."
d. If we increase number of *cis* double bonds in a fatty acid it results in variety of possible fatty acids like arachidonic acid, with four cis double bonds, has "kinks" or a U shape.
e. *Trans*- double bonds alter these spatial relationships.
f. As we increase it will further increase the melting points of even-numbered-carbon fatty acids increase with chain length and decrease according to unsaturation.



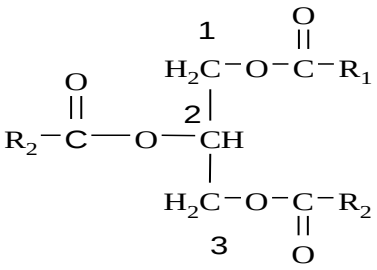
Geometric isomerism of fatty acids (oleic and elaidic acids)



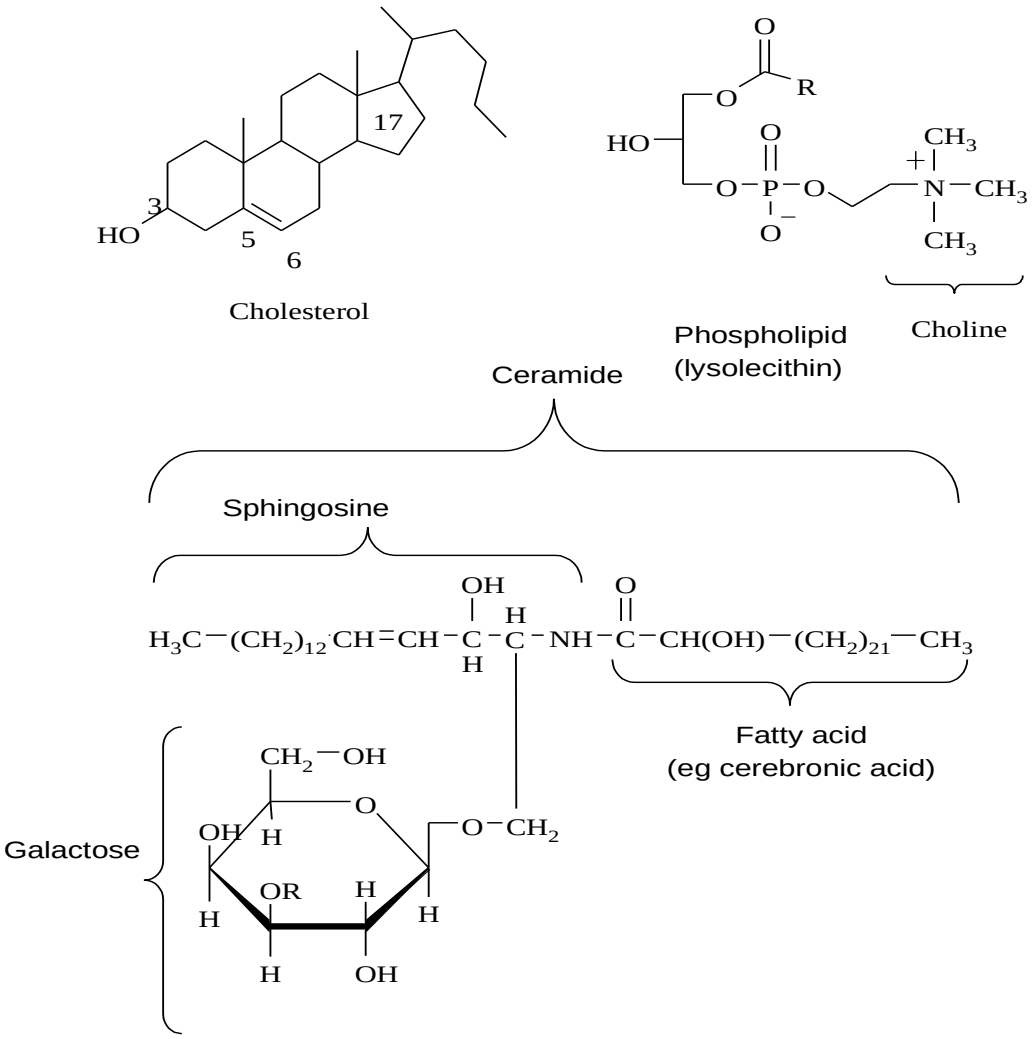
6.1.5 Structures of Some Common Classes of Lipid:



Oleic acid



Triacylglycerol



Glycolipids (galactosylceramide)

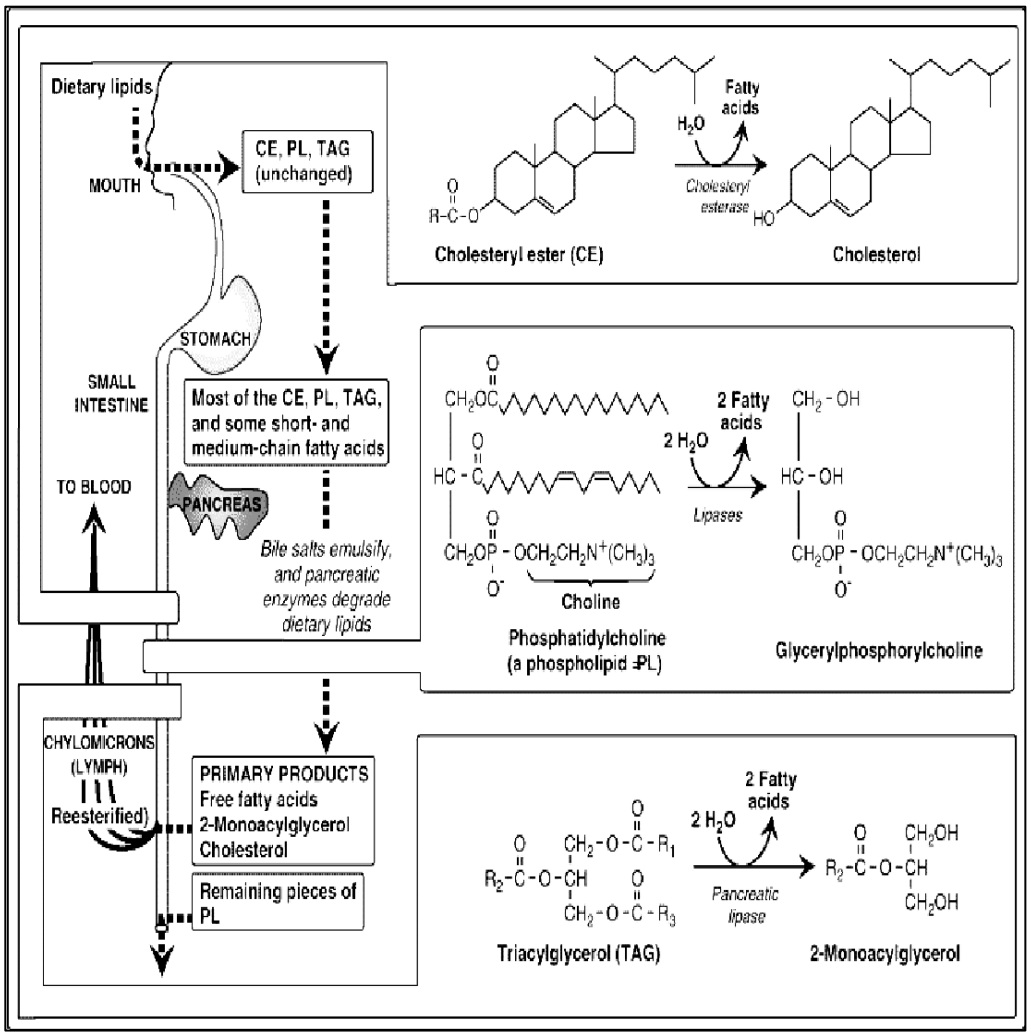
Note by: Naturally, occurring unsaturated vegetable oils have almost all *Cis* bonds, but using oil for frying causes some of the *Cis* bonds to convert to Trans bonds.

Fatty acids with Tran's bonds are carcinogenic.

6.1.6 Digestion, Absorption, Secretion, and Utilization of Dietary Lipids:

The common each day intake of lipids for an adult is about 81 g, of which more than 90% is usually triacylglycerol (TAG-triglyceride).

The remainder of the dietary lipids consists principally cholesterol, cholesteryl esters, phospholipids, and unesterified ("free") fatty acids.



6.2 Emulsification of Lipid in the Small Intestine:

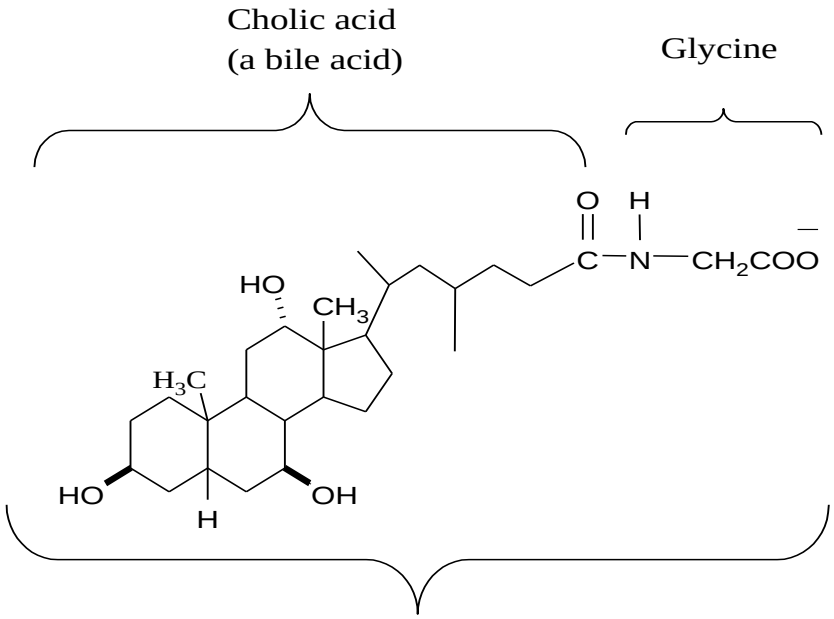
Emulsification of lipids a significant process occurs in the duodenum. Bile salts generally carry out emulsification, and mechanical mixing due to peristalsis.

Bile salts, made in the liver and stored in the gallbladder, are derivatives of cholesterol.

Bile salts, synthesized in the liver and stored in the gallbladder, are derivatives of cholesterol.

They consist of a sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage.

These emulsifying agents interact with the dietary lipid particles and the aqueous duodenal contents, thereby stabilizing the particles as they become smaller, and preventing them from coalescing.



Glycocholic acid
(a bile salt)

6.2.1 Use of Dietary Lipids by the Tissues:

The site for breakdown of the chylomicrons which contains is the skeletal muscle and adipose tissue and some other sites viz. heart, lung, kidney, and liver.

Triacylglycerol in chylomicrons is destroyed to free fatty acids and glycerol by *lipoprotein lipase*. This enzyme is produced primarily in adipocytes and muscle cells.

The free fatty acids derived from the hydrolysis of TAG be transported in the blood in association with serum albumin and they are taken up by cells where it oxidize fatty acids to produce energy.

Glycerol is also synthesized and released from TAG later is used almost exclusively by the liver to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate.

Note by: *Chylomicrons* are transport proteins for dietary lipids from the intestines to other locations in the body.

Chylomicrons are one of the 5 major groups of lipoproteins which enable fats and cholesterol to move within the water based solution of the blood stream.

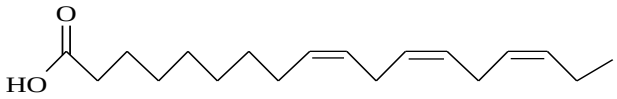
Chylomicrons transport exogenous lipids to liver, adipose, cardiac and skeletal muscle tissue.

6.2.2 Essential Fatty Acids:

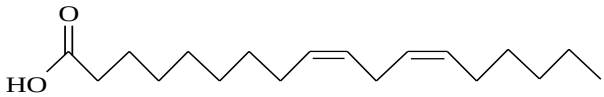
Two fatty acids are dietary basic in humans

a. **Linoleic acid,** the precursor for arachidonic acid, the substrate for prostaglandin synthesis.
b. **α-linolenic** acid is the precursor for growth and development.

It is one of the essential fatty acid and its deficiency can result in a scaly dermatitis, as well as visual and neurologic abnormalities.



linolenic acid



linoleic acid

6.2.3 Fatty Acid and Triacylglycerol Metabolism:

Fatty acids are generally considered as free and are found as fatty acyl esters in more complex molecules, such as triacylglycerols. During fasting low levels of free fatty acids occur in all tissues, but substantial amounts can sometimes be found in the plasma.

The free fatty acids can be used as energy resources through oxidation by many tissues particularly liver and muscle to provide energy.

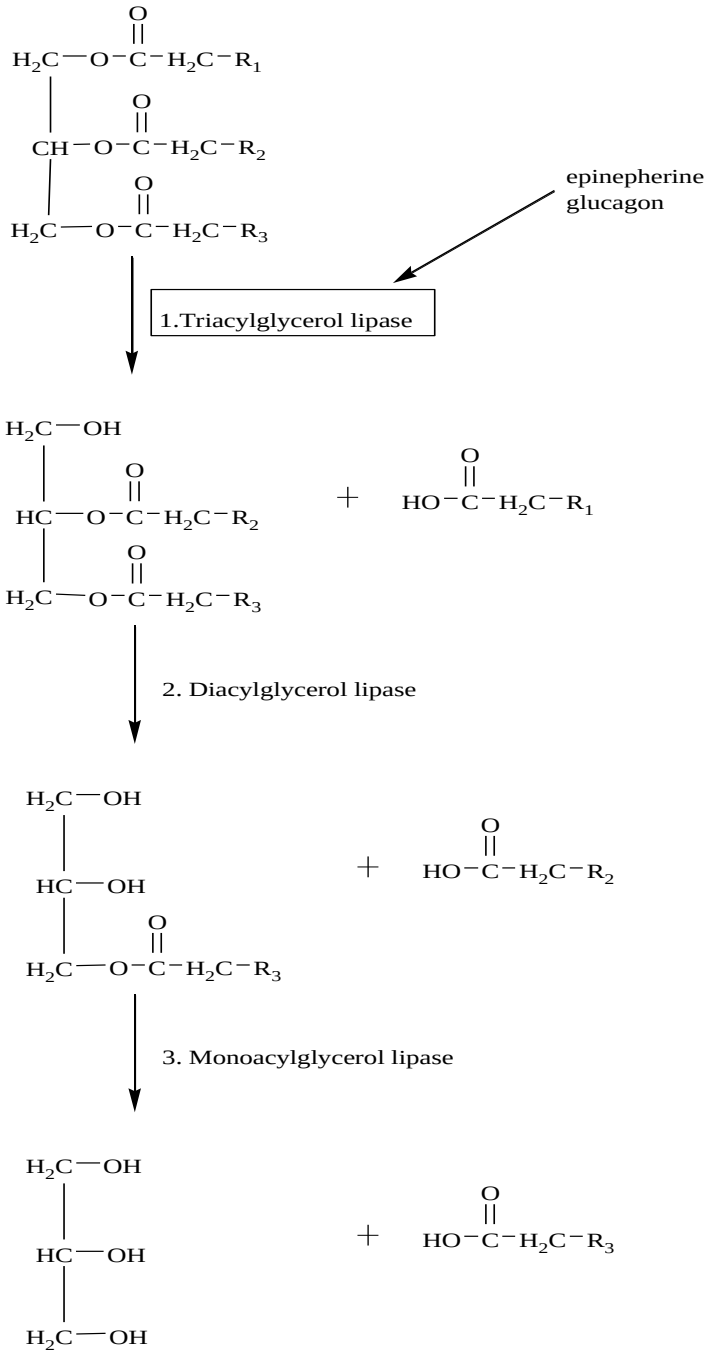
These are also structural components of membrane lipids, such as phospholipids and glycolipids.

Fatty acids are also precursors of the hormone-like prostaglandins.

Esterified fatty acids, in the form of triacylglycerols stored in adipose cells, serve as the major energy reserve of the body.

6.2.4 Breakdown of Triglyceride is facilitated by three Enzymes:

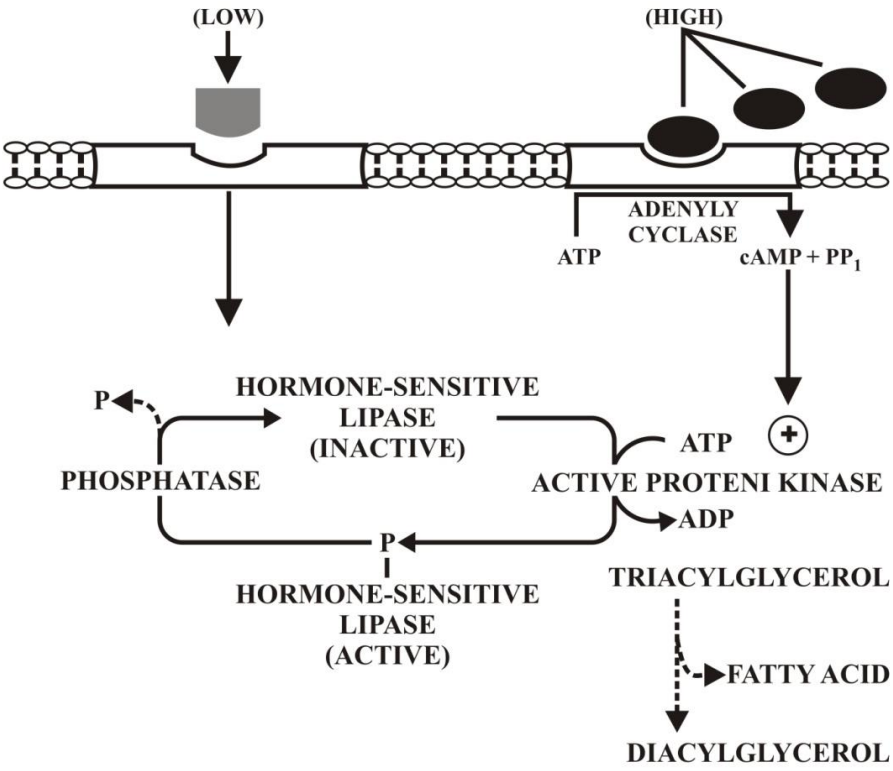
a. Triacylglycerol lipase (which is activated by epinephrine)
b. Diacyclglycerol lipase
c. Monoacylglycerol lipase



6.2.5 Release of Fatty Acids from TAG:

The stored fat mobilization requires the hydrolytic release of fatty acids and glycerol from their TAG form. Hormone-sensitive lipase initiated this process, which removes a fatty acid from carbon 1 and/or carbon 3 of the TAG. Additional lipases specific for diacylglycerol or monoacylglycerol remove the remaining fatty acids.

a. ***Hormonal Control of Lipolysis:*** The breakdown of triglycerides by lipases is under hormonal control. The major enzymes concerned are epinephrine, glucagon and insulin. Epinephrine and glucagon promote the breakdown of fat (lipolysis) while insulin inhibits fat breakdown.



6.2.6 Hormonal Regulation of Triacylglycerol Degradation:

3', 5'-cyclic AMP (cAMP)–dependent protein kinase phosphorylation activates hormone-sensitive lipase (HSL).

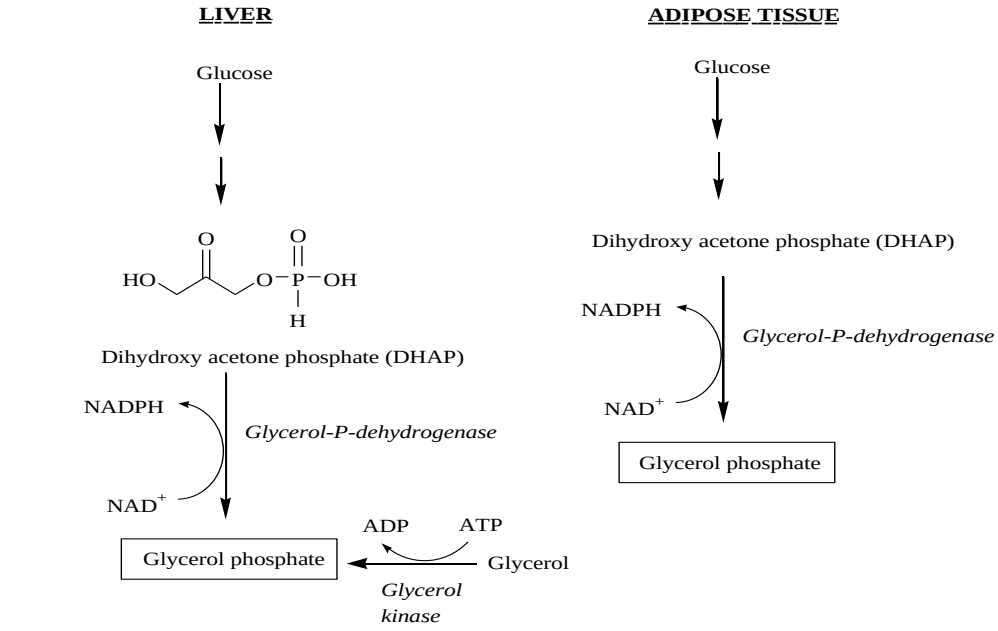
3', 5'-Cyclic AMP is produced in the adipocyte by several hormones like as epinephrine or glucagon binds to receptors on the cell membrane, and activates adenylyl cyclase.

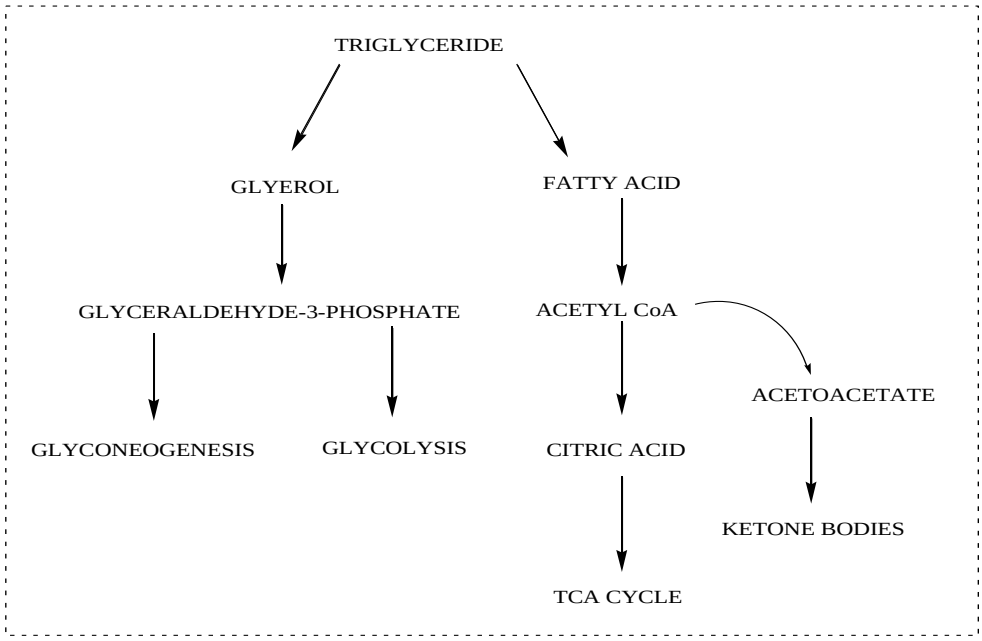
When TAG degradation is turned on the fatty acid synthesis is turned off. In the presence of high plasma levels of insulin and glucose, HSL is dephosphorylated, and becomes inactive.

The glycerol released during TAG degradation cannot be metabolized by adipocytes because they apparently lack glycerol kinase. Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated.

The resulting glycerol phosphate can be used to form **TAG** in the liver, or can be converted to **by** the glycerol phosphate dehydrogenase.

6.2.7 Various Pathway Generations during Triacylglycerol Degradation:





6.3 β-Oxidation of Fatty Acids:

β-oxidation is the major pathway for catabolism of even-numbered saturated fatty acids is a mitochondrial pathway. In which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and $FADH_2$.

In β-oxidation, the fatty acid is broken down to release acetyl-CoA. This process involves 4 main steps:

a. Dehydrogenation
b. Hydration
c. Oxidation
d. Thiolysis

Beta-oxidation of fatty acids takes place in the mitochondrial matrix for the most part.

However, fatty acids have to be activated for degradation by ***coenzyme A*** by forming a **fatty acyl-CoA thioester.**

For short and medium length fatty acids, they undergo this reaction in the mitochondria.

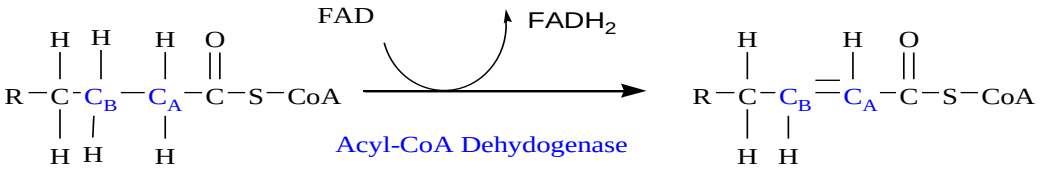
The long chain fatty acids can't go through the membrane though, so this reaction occurs at the outer mitochondrial membrane.

The final fatty acid products are **acetyl-CoA** for the even numbered fatty acids (without double bonds), and for those with an odd number of carbons, it is **3-carbon propionyl-CoA.**

a. Beta-Oxidation of Fatty Acids (even chain):

- **Dehydrogenation (Acyl-CoA Dehydrogenase):** This first reaction is the oxidation of the C_a-C_b bond.

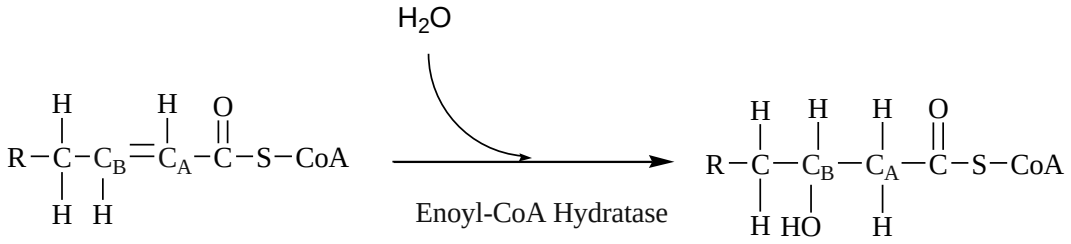
It is catalyzed by *acyl-CoA dehydrogenases.* This catalyst is a family of three soluble matrix enzymes. These enzymes carry noncovalently bound FAD that is reduced during the oxidation of the fatty acid.



Fattyacyl-CoA *trans*-2-Enoyl-CoA

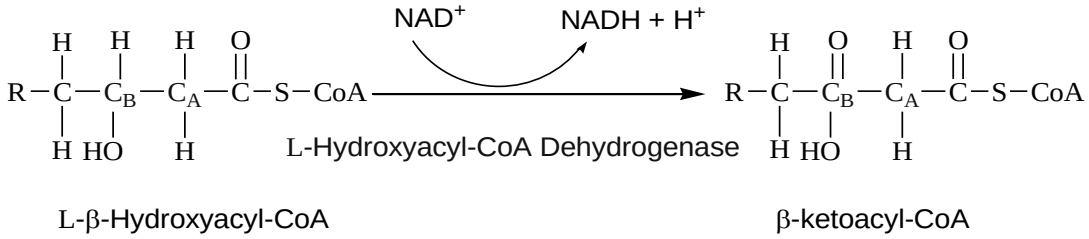
- **Hydration (Enoyl-CoA Hydratase):** In this pathway is one in which water is added across the new double bond to make hydroacyl-CoA. The catalyst in this reaction is Enoyl-CoA hydratase.

This is also called a crotonase and it converts trans-enoyl-CoA to L-B-Hydroxyacyl-CoA. This reaction would be classified as a hydration reaction because you are adding water.

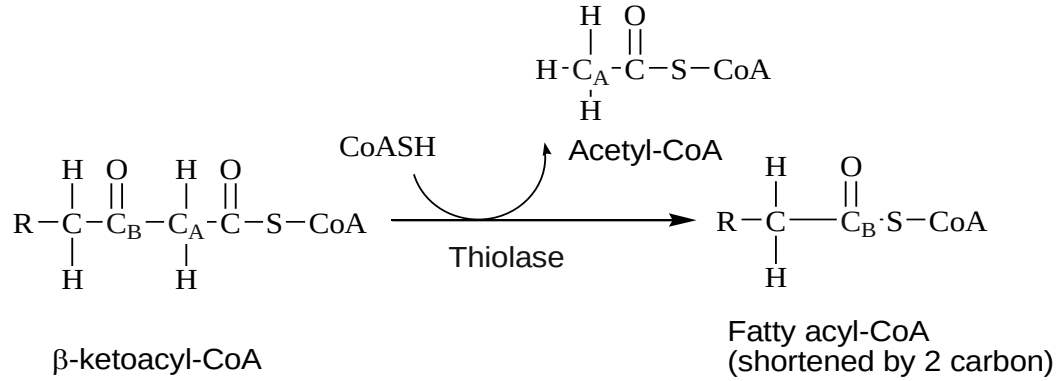


- **Oxidation (L-Hydroxyacyl-CoA Dehydrogenase):** Here the oxidation of the hydroxyl group at the beta position which forms a beta-ketoacyl-CoA derivative and it is catalyzed by L-Hydroxyacyl-CoA Dehydrogenase.

This enzyme needs to have NAD+ as a coenzyme and the NADH produced represents metabolic energy because for every NADH produced, it drives the synthesis of 2.5 molecules of ATP in the electron transport pathway. So, this reaction is classified as an oxidation reaction.



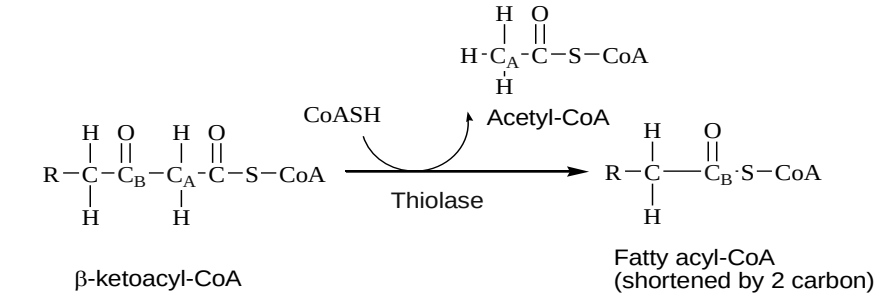
- **Thiolysis:** This is the final reaction of this pathway and thiolase catalyzed this reaction. This reaction cleaves the β-ketoacyl-CoA. The products of this reaction are an acetyl-CoA and a fatty acid that has been shortened by two carbons. So, this reaction is classified as a cleavage reaction.

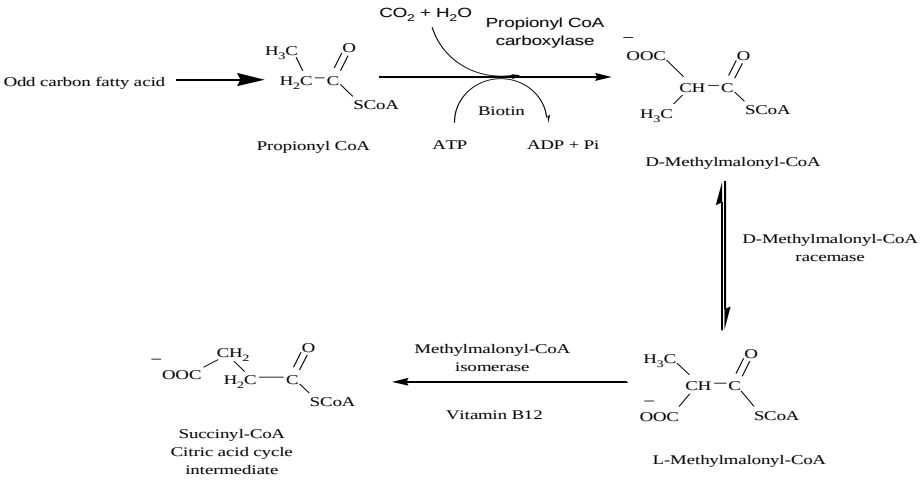


6.3.1 Repetition of the Beta Oxidation Cycle:

The shortened fatty acyl-CoA that was the product of the last reaction now goes through another beta-oxidation cycle. This keeps happening until eventually you wind up with two molecules of acetyl-CoA in the final step. This acetyl-CoA is then available to be further metabolized in the TCA cycle, or it can be used as a substrate in amino acid biosynthesis. It cannot be used as a substrate for gluconeogenesis. Beta oxidation of fatty acid with an odd number of carbons. Chains with an odd-number of carbons are oxidized in the same manner as even-numbered chains, but the final products are propionyl CoA and acetyl CoA. Propionyl CoA is converted into succinyl CoA (which is an intermediate in the citric acid cycle) in a reaction that involves Vitamin B_{12}. Succinyl CoA can then enter the citric acid cycle. Because it cannot be completely metabolized in the citric acid cycle, the products of its partial reaction must be removed in a process called cataplerosis.

This allows regeneration of the citric acid cycle intermediates, possibly an important process in certain metabolic diseases. Animals cannot make glucose from even carbon fatty acids. The only scope for glucose synthesis from fatty acids is from the propionyl CoA left behind after the beta-oxidation of odd carbon fatty acids.





6.3.2 Energy yield during β-Oxidation of Fatty Acids:

The ATP yield for every oxidation cycle is 14 ATP, broken down as follows:

1 FADH2 x 1.5 ATP = 1.5 ATP
1 NADH x 2.5 ATP = 2.5 ATP
1 acetyl CoA x 10 ATP = 10 ATP

For an even-numbered saturated fat (C_2n), n - 1 oxidations are necessary and the final process yields an additional acetyl CoA. In addition, two equivalents of ATP are lost during the activation of the fatty acid.

Therefore, the total ATP yield can be stated as: (n - 1) * 14 + 10 – 2= No. of ATP For instance, the ATP yield of Palmitate (C_{16}, n = 8) is:

(8 - 1) * 14 + 10 – 2=106 ATP

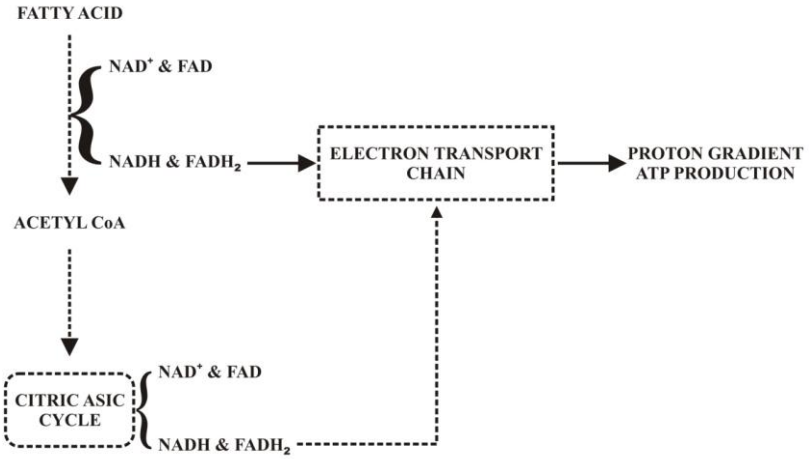
Or,

7 FADH2 x 1.5 ATP = 10.5 ATP

7 NADH x 2.5 ATP = 17.5 ATP

8 acetyl CoA x 10 ATP = 80 ATP

ATP equivalent used during activation = -2



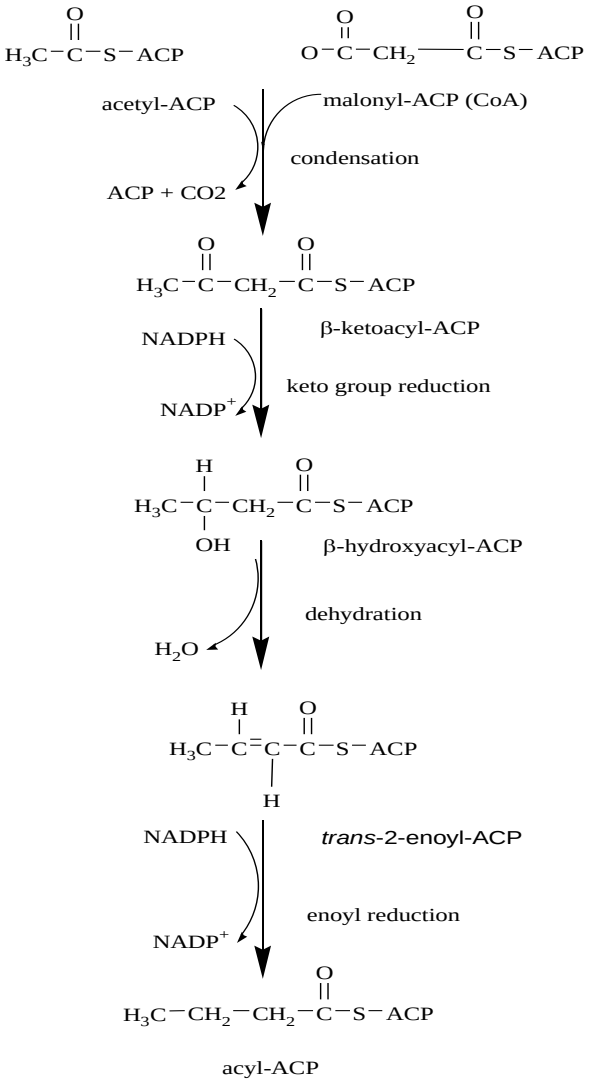
6.3.3 Biosynthesis of Fatty Acids:

Fatty acid synthesis is the creating of fatty acids from acetyl-CoA and malonyl-CoA precursors through action of enzymes called fatty acid synthases. It is an important part of the lipogenesis process, which - together with glycolysis stands behind creating fats from blood sugar in living organisms. Synthesis takes place in the cytosol. In humans, fatty acids are predominantly formed in the liver and lactating mammary glands, and, to a lesser extent, the adipose tissue. Most acetyl-CoA is formed from pyruvate by pyruvate dehydrogenase

in the mitochondria. Acetyl- CoA produced in the mitochondria is condensed with oxaloacetate by citrate synthase to form citrate, which is then transported into the cytosol and broken down to yield acetyl-CoA and oxaloacetate by ATP citrate lyase. Oxaloacetate in the cytosol is reduced to malate by cytoplasmic malate dehydrogenase, and malate is transported back into the mitochondria to participate in the Citric acid cycle.

The Process Involves 4 Main Steps:

a. Condensation,
b. Reduction,
c. Dehydration
d. Reduction



Acyl carrier protein (ACP): The acyl carrier protein (ACP) is an important component in both fatty acid and polyketide biosynthesis.

Table 6.1: Differences between Fatty Acid Degradation and Synthesis:

Characteristic	Degradation	Synthesis
Location	Mitochondrial Matrix	Cytosol
Activated intermediates	Thioesters of CoA	Thioesters of ACP
Process	2-Carbon fragments removed as acetyl CoA	2-Carbon elongation using malonyl CoA
Direction	Starts at carboxyl end	Starts at methyl end
Redox reaction cofactors	FAD/$FADH_2$ and NAD^+/NADH	NADP+/NADPH
Major tissue site	Muscle and liver	Liver
Hormonal regulation	Low insulin / glucagon ratio	High insulin/glucagon ratio
Activator	FFA generated by hormone-sensitive lipase	Citrate
Inhibitor	Malonyl CoA (inhibits carnitine acyl transferase)	Fatty acyl CoA (inhibits acetyl CoA
		carboxylase)

6.4 Ketogenesis:

a. The process by which ketone bodies are produced as a result of fatty acid breakdown is called as Ketogenesis.
b. The synthesis of ketone bodies occurs in response to low glucose levels in the blood, and after exhaustion of cellular carbohydrate stores, such as glycogen. They are mainly produced in the mitochondria of liver cells. The production of ketone bodies is then initiated to make available energy that is stored as fatty acids.
c. Besides its role in the synthesis of ketone bodies, HMG-CoA is also an intermediate in the synthesis of cholesterol.
d. The three ketone bodies are:

Acetoacetate, which, if not oxidized to form usable energy, is the source of the two other ketone bodies below

Acetone, which is not used as an energy source, but is instead exhaled or excreted as waste

β-hydroxybutyrate, which is not, in the technical sense, a ketone according to IUPAC nomenclature.

6.4.1 Regulation:

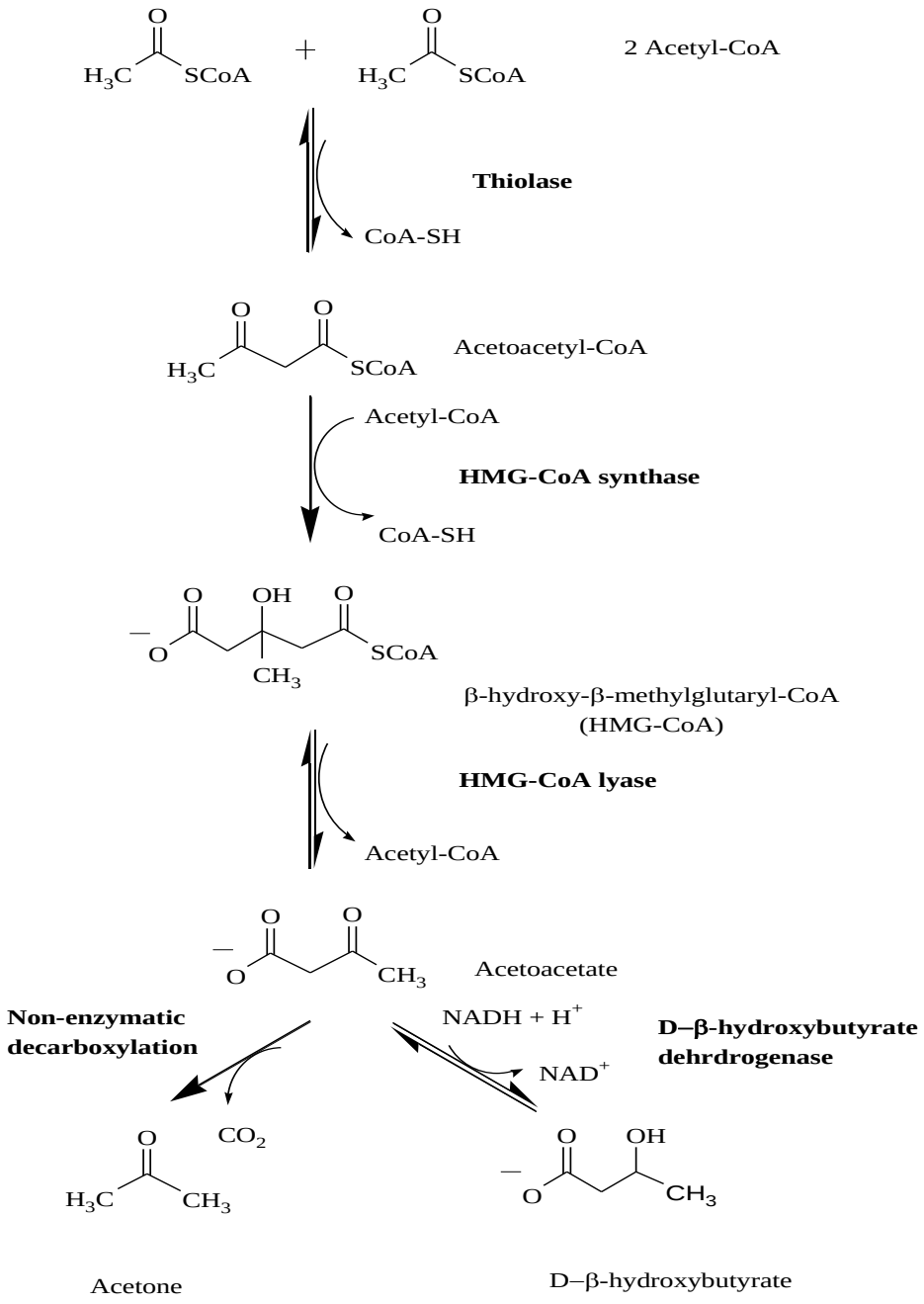
Ketogenesis may or may not occur, depending on levels of available carbohydrates in the cell or body. This is closely related to the paths of acetyl-CoA:

a. When the body has ample carbohydrates available as energy source, glucose is completely oxidized to CO_2; acetyl-CoA is formed as an intermediate in this process, first entering the citric acid cycle followed by complete conversion of its chemical energy to ATP in oxidative phosporylation.
b. When the body has excess carbohydrates available, some glucose is fully metabolized, and some of it is stored by using acetyl-CoA to create fatty acids. (CoA is also recycled here.)
c. When the body has no free carbohydrates available, fat must be broken down into acetyl-CoA in order to get energy.

6.4.2 Pathology:

a. Ketone bodies are produced at moderate levels in everyone's bodies, such as during sleep and other times when no carbohydrates are available.
b. However, when ketogenesis is happening at higher-than-normal levels, the body is said to be in a state of ketosis.
c. Both acetoacetate and beta-hydroxybutyrate are acidic, and, if levels of these ketone bodies are too high, the pH of the blood drops, resulting in ketoacidosis.

Ketoacidosis is known to occur in untreated Type I diabetes (diabetic ketoacidosis) and in alcoholics after prolonged binge-drinking without intake of sufficient carbohydrates (alcoholic ketoacidosis).



Ketogenesis Pathway

6.5 Ketoacidosis:

a. Ketoacidosis is a metabolic state connected with high concentrations of ketone bodies, formed by the breakdown of fatty acids and the deamination of amino acids. The two common ketones produced in humans are acetoacetic acid and *β-hydroxybutyrate.*

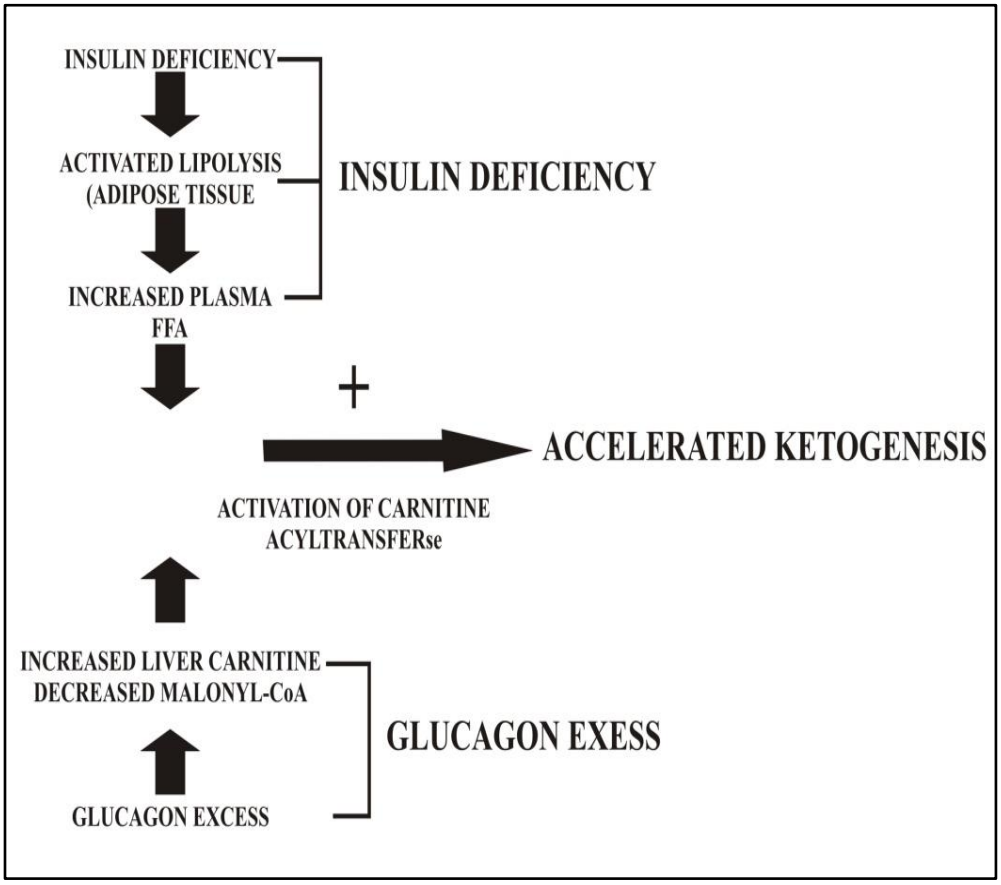
b. In ketoacidosis, the body fails to adequately regulate ketone production causing such a severe accumulation of keto acids that the pH of the blood is substantially decreased. In extreme cases ketoacidosis can be fatal.
c. Ketoacidosis occurs when the body is producing large quantities of ketone bodies via the metabolism of fatty acids (ketosis) and the body is producing insufficient insulin to slow this production.
d. The excess ketone bodies can significantly acidify the blood.
e. There are two common types of Ketoacidosis i.e. diabetic and alcoholic ketoacidosis.

- In diabetic patients, ketoacidosis is usually accompanied by insulin deficiency, hyperglycemia, and dehydration.

Particularly in type 1 diabetics the lack of insulin in the bloodstream prevents glucose absorption and can cause unchecked ketone body production

- In alcoholic ketoacidosis, alcohol causes dehydration and blocks the first step of gluconeogenesis.

The body is unable to synthesize enough glucose to meet its needs, thus creating an energy crisis resulting in fatty acid metabolism, and ketone body formation.



6.5.1 Ketone Bodies:

a. Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney.
b. They are used as a source of energy in the heart and brain. In the brain, they are a vital source of energy during fasting.
c. The three endogenous ketone bodies are acetone, acetoacetic acid, and beta-hydroxybutyric acid, - although beta-hydroxybutyric acid is not technically a ketone but a carboxylic acid.
d. Ketone bodies -are produced from acetyl-CoA (ketogenesis) mainly in the mitochondrial matrix.
e. Ketone bodies can be used for energy. Ketone bodies are transported from the liver to other tissues, where acetoacetate and beta-hydroxybutyrate can be reconverted to acetyl-CoA to produce energy, via the citric acid cycle.
f. When even larger amounts of ketone bodies accumulate such that the blood's pH is lowered to dangerously acidic levels, this state is called ketoacidosis.

6.5.2 Ketonuria:

a. Ketonuria is a medical condition in which ketone bodies are present in the urine.
b. It is seen in conditions in which the body produces excess ketones as an alternative source of energy. It is seen during starvation or more commonly in type I diabetes mellitus. Production of ketone bodies is a normal response to a shortage of glucose, meant to provide an alternate source of fuel from fatty acids.
c. Causes of ketosis and ketonuria

- Metabolic abnormalities such as diabetes, renal glycosuria, or glycogen storage disease.
- Dietary conditions such as starvation, fasting, high protein, ior low carbohydrate diets, prolonged vomiting, and anorexia.
- Conditions in which metabolism is increased, such as hyperthyroidism, fever, pregnancy or lactation.

d. In nondiabetic persons, ketonuria may occur during acute illness or severe stress. Approximately 15% of hospitalized patients may have ketonuria, even though they do not have diabetes.

e. In the nondiabetic patient, ketonuria reflects a reduced carbohydrate metabolism and an increased fat metabolism.

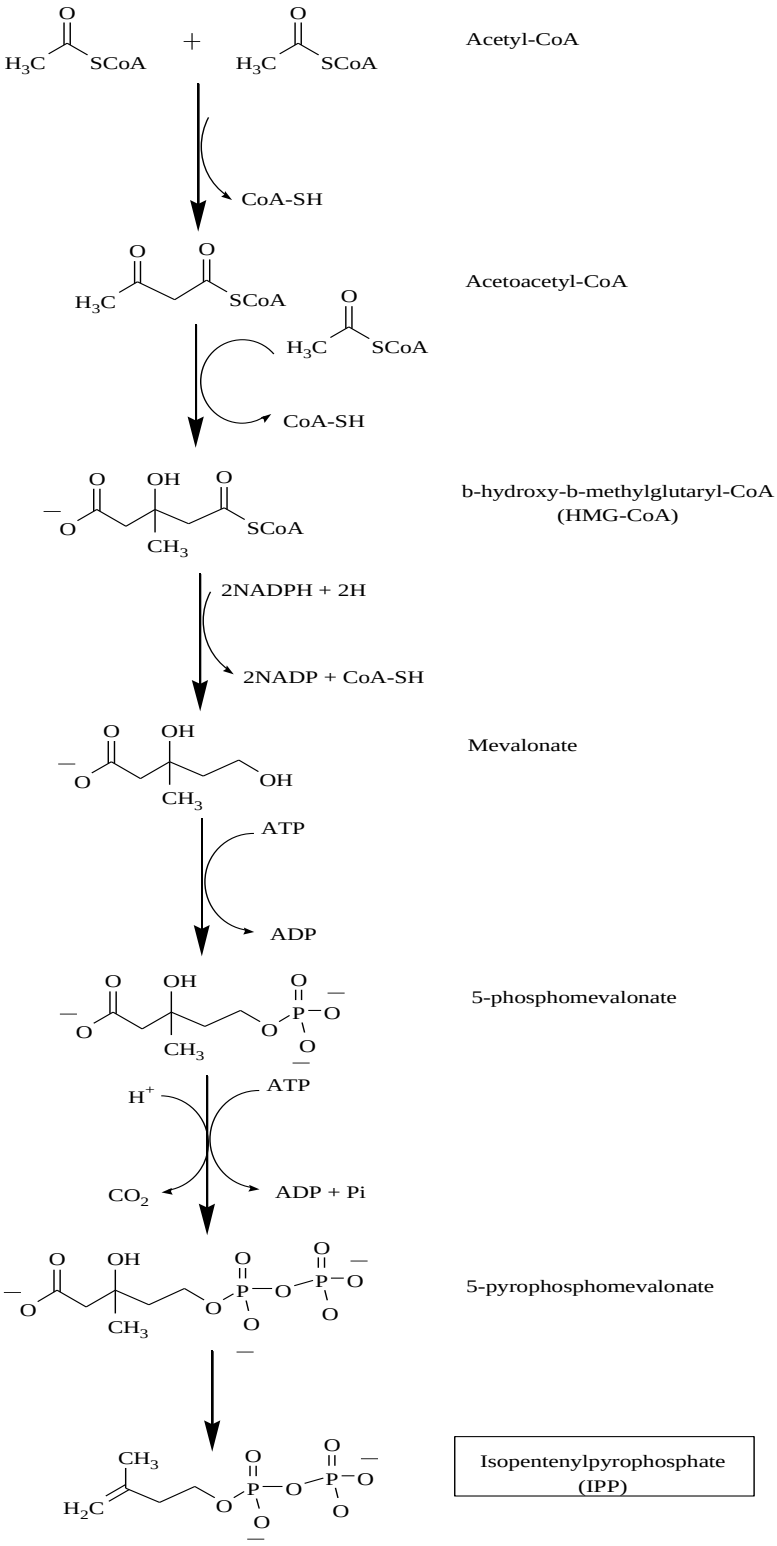
6.6 Cholesterol Synthesis, Transport and Excretion:

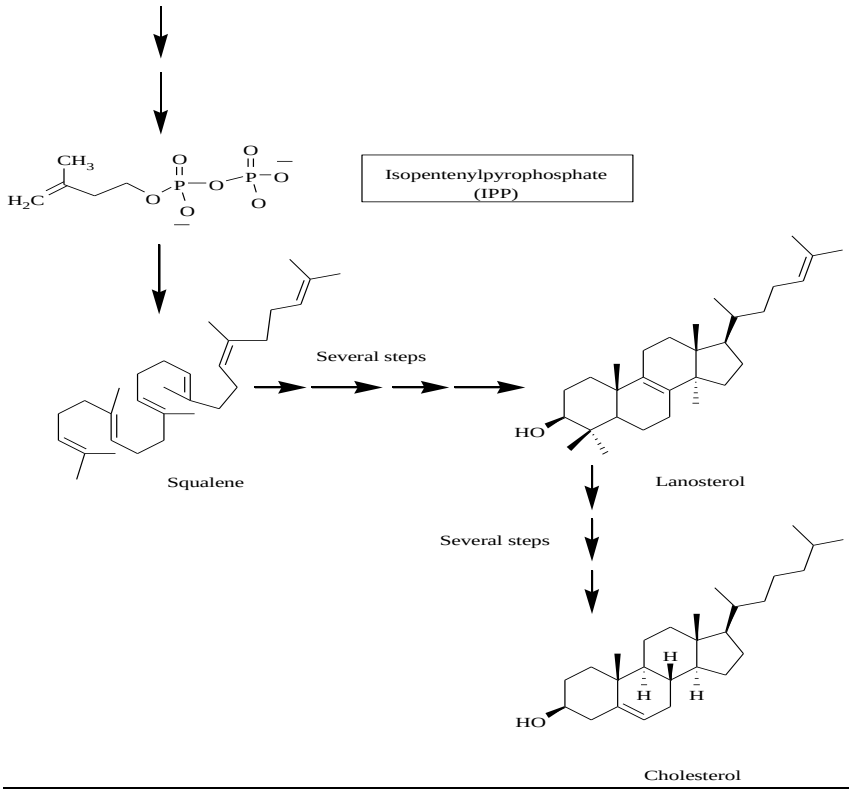
a. Cholesterol is present in tissues and in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholesteryl ester.
b. b. Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins.
c. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D.

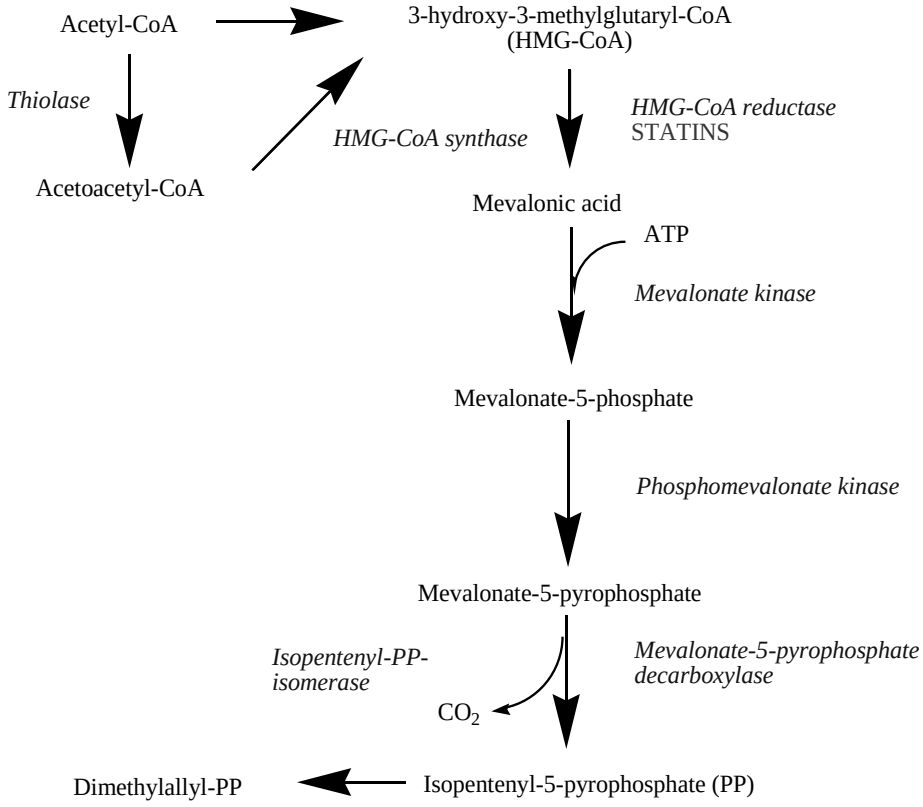
d. Plasma low-density lipoprotein (LDL) is the vehicle of uptake of cholesterol and cholesteryl ester into many tissues. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion to bile acids in the process known as reverse cholesterol transport.
e. Cholesterol is a major constituent of gallstones. However, its chief role in pathologic processes is as a factor in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary and peripheral vascular disease.
f. Biosynthesis of cholesterol: Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA.
g. Biosynthesis of cholesterol in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. The process has five major steps:

- Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
- HMG-CoA is converted to mevalonate
- Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO_2
- IPP is converted to squalene and
- Then Squalene is converted to cholesterol.

6.7 Mevalonate" Pathway to IPP Synthesis




Isopentenylpyrophosphate
(IPP)
Several steps
Squalene
Lanosterol
Several steps
Cholesterol


Acetyl-CoA
3-hydroxy-3-methylglutaryl-CoA
(HMG-CoA)
Thiolase
HMG-CoA synthase
HMG-CoA reductase
STATINS
Acetoacetyl-CoA
Mevalonic acid
ATP
Mevalonate kinase
Mevalonate-5-phosphate
Phosphomevalonate kinase
Mevalonate-5-pyrophosphate
Isopentenyl-PP-
isomerase
Mevalonate-5-pyrophosphate
decarboxylase
CO2
Dimethylallyl-PP
Isopentenyl-5-pyrophosphate (PP)

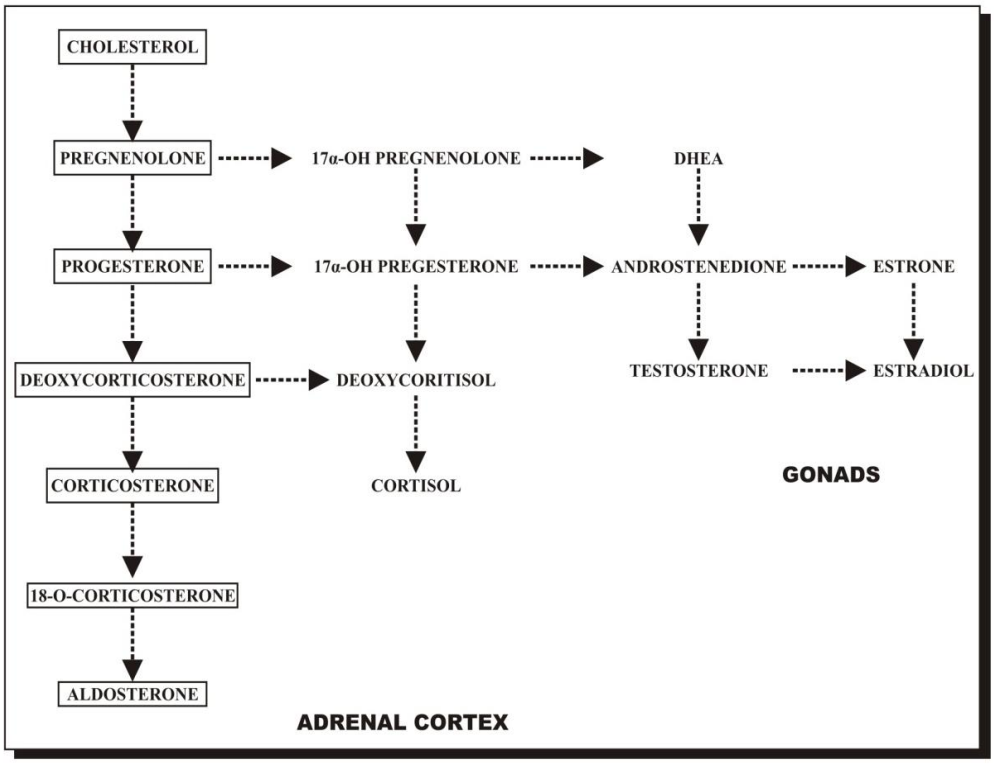
6.8 Steroidogenesis:

a. It is the process wherein desired forms of steroids are generated by transformation of other steroids.

The pathways of human steroidogenesis are shown in the figure.

6.8.1 Products of Steroidogenesis Include:

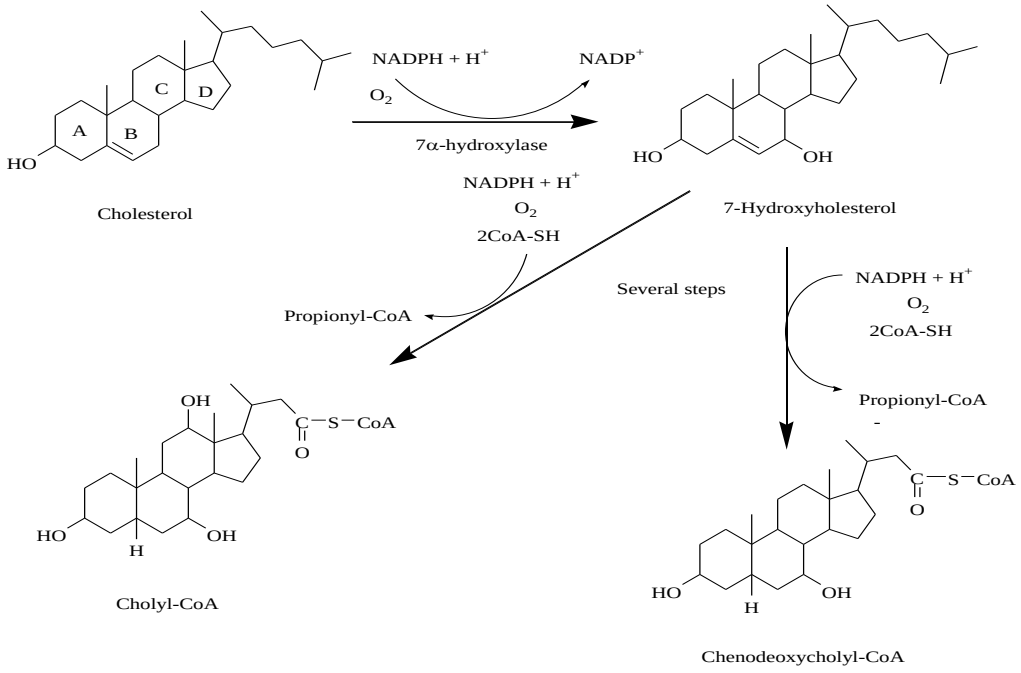
- androgen
- testosterone
- estrogens and progesterone
- corticoids
- cortisol
- aldosterone



6.8.2 Elimination of Steroids:

A. Steroids are mainly oxidized by cytochrome P450 oxidase enzymes, such as CYP3A4. These reactions introduce oxygen into the steroid ring and allow the structure to be broken up by other enzymes, to form bile acids as final products. These bile acids can then be eliminated through secretion from the liver in the bile.

The end products of cholesterol utilization are the bile acids, synthesized in the liver. Synthesis of bile acids is one of the predominant mechanisms for the excretion of excess cholesterol. However, the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess dietary intake of cholesterol.



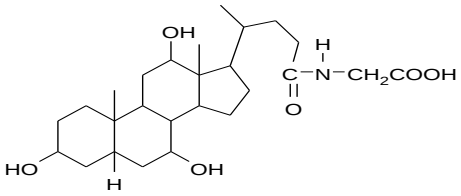
B. the most abundant bile acids in human bile are **chenodeoxycholic acid** (45%) and **cholic acid** (31%). These are referred to as the primary bile acids. Within the intestines the **primary bile acids** are acted upon by bacteria and converted to the **secondary bile acids,** identified as **deoxycholate** (from cholate) and **lithocholate** (from chenodeoxycholate). Both primary and secondary bile acids are reabsorbed by the intestines and delivered back to the liver via the portal circulation.

C. Within the liver the carboxyl group of primary and secondary bile acids is conjugated via an amide bond to either glycine or taurine before their being re-secreted into the bile canaliculi. These conjugation reactions yield **glycoconjugates** and **tauroconjugates,** respectively. The bile canaliculi join with the bile ductules, which then form the bile ducts. Bile acids are carried from the liver.

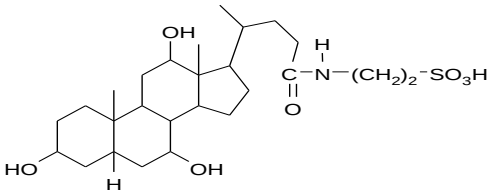
D. through these ducts to the gallbladder, where they are stored for future use. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids.

In the gut the glycine and taurine residues are removed and the bile acids are either excreted (only a small percentage) or reabsorbed by the gut and returned to the liver.

This process of secretion from the liver to the gallbladder, to the intestines and finally reabsorbtion is termed the enterohepatic circulation.



Glycocholic acid



Taurocholic acid

6.9 Atherosclerosis:

a. Atherosclerosis is a disease in which plaque builds up on the insides of arteries.
b. It is a syndrome affecting arterial blood vessels. It is a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by low density (especially small particle) lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL). It is commonly referred to as a "hardening" or "furring" of the arteries. It is caused by the formation of multiple plaques within the arteries.
c. The atheromatous plaque is divided into three distinct components:

- The atheroma ("lump of porridge), which is the nodular accumulation of a soft, flaky, yellowish material at the center of large plaques, composed of macrophages nearest the lumen of the artery
- Underlying areas of cholesterol crystals
- Calcification at the outer base of older/more advanced lesions.

d. Atherosclerosis can affect any artery in the body, including arteries in the heart, brain, arms, legs, and pelvis. As a result, different diseases may develop based on which arteries are affected.

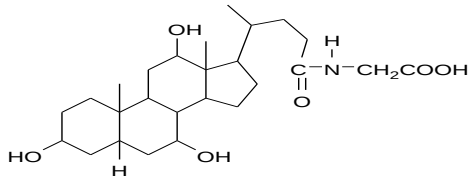
- **Coronary artery disease**: (CAD). This is when plaque builds up in the coronary arteries. These arteries supply oxygen-rich blood to your heart. When blood flow to your heart is reduced or blocked, it can lead to chest pain and heart attack. CAD also is called heart disease, and it's the leading cause of death in the United States.
- **Carotid artery disease:** This happens when plaque builds up in the carotid arteries. These arteries supply oxygen-rich blood to your brain. When blood flow to your brain is reduced or blocked, it can lead to stroke.
- **Peripheral arterial disease (PAD):** This occurs when plaque builds up in the major arteries that supply oxygen-rich blood to the legs, arms, and pelvis. When blood flow to these parts of your body is reduced or blocked, it can lead to numbness, pain, and sometimes dangerous infections.

6.9.1 Symptoms of Atherosclerosis:

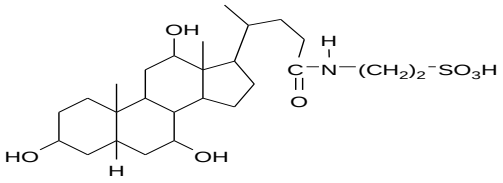
a. Unfortunately, atherosclerosis produces no symptoms until the damage to the arteries is severe enough to restrict blood flow.

b. Restriction of blood flow to the heart muscle due to atherosclerosis can cause angina pectoris or a myocardial infarction (a heart attack).
c. Restriction of blood flow to the muscles of the legs causes' intermittent claudicating (pains in the legs brought about by walking and relieved by rest).

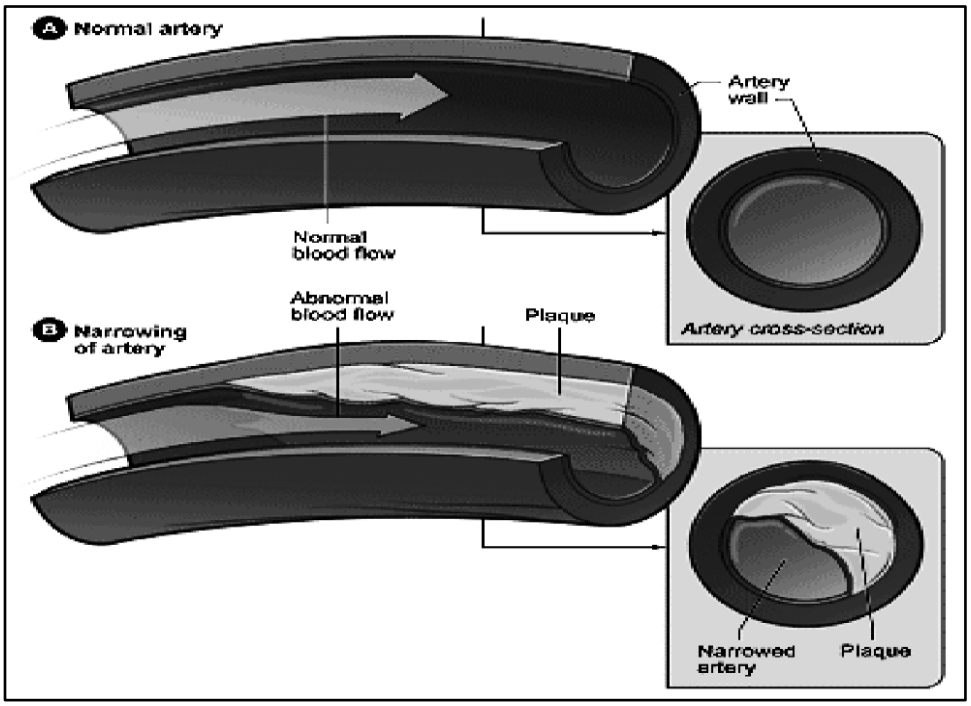
Narrowing of the arteries supplying blood to the brain may cause transient ischemic attacks (symptoms and signs of a stroke lasting less than 24 hours) and episodes of dizziness, or ultimately, to a stroke itself.



Glycocholic acid



Taurocholic acid



6.9.2 Treatment of Atherosclerosis:

a. Medication is unsatisfactory for treating atherosclerosis, since the damage has already been done.
b. Anticoagulant drugs have been used to try to minimize secondary clotting and embolus formation.
c. Vasodilator drugs are helpful in providing symptom relief, but are of no curative value.
d. Surgical treatment is available for those unresponsive to medical treatment or in certain high-risk situations.

e. Balloon angioplasty can open up narrowed vessels and promote an improved blood supply.
f. The blood supply to the heart can also be restored by coronary artery bypass surgery.

Ketolysis: The splitting up of ketone bodies.

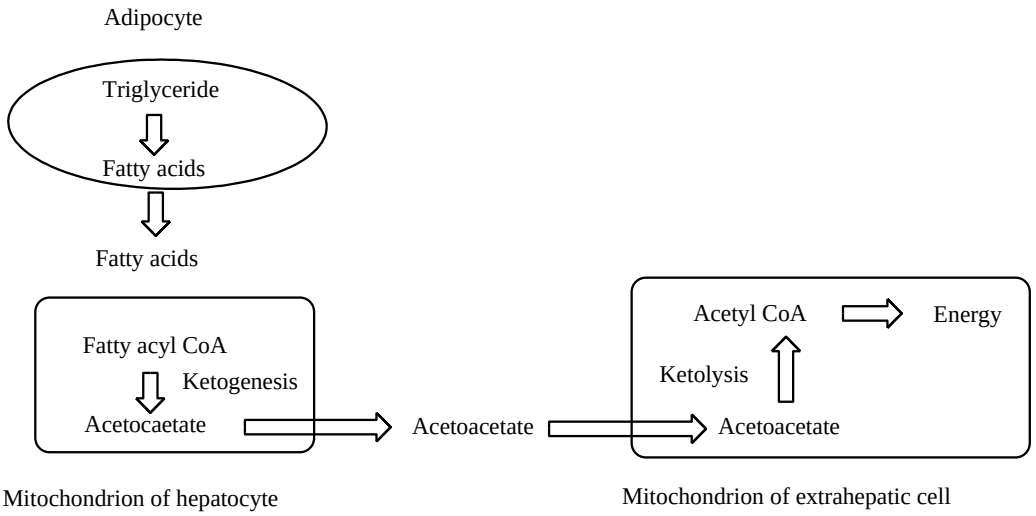
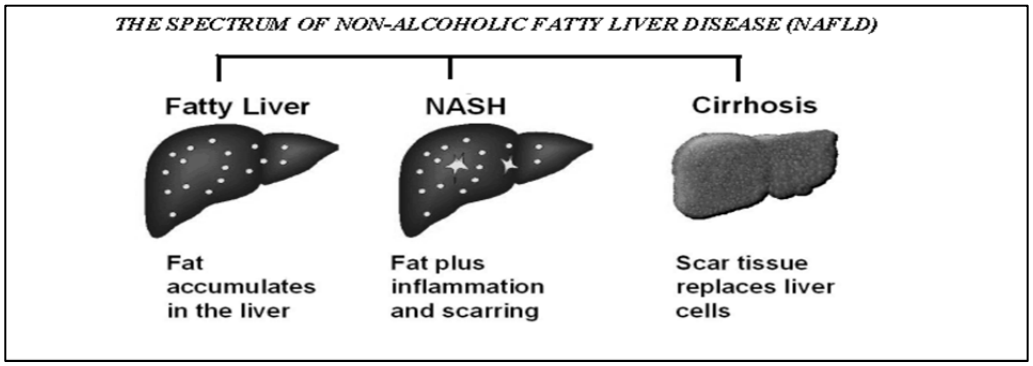


Diagram of ketone body metabolism

6.10 Fatty Liver:

a. It is also known as ***fatty liver disease*** **(FLD),** is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of ***steatosis*** (i.e. abnormal retention of lipids within a cell).
b. ***Causes:*** *Fatty liver is commonly associated with alcohol or metabolic syndrome (diabetes, hypertension, obesity and dyslipidemia)*
c. Diagnosis of Fatty Liver: in routine blood screening or images of the liver obtained by an ultrasound test, CT (computed tomography) scan, or MRI (magnetic resonance imaging) may suggest the presence of a fatty liver or **liver biopsy, in** which a small sample of liver tissue is obtained through the skin and analyzed under the microscope
d. The treatment of fatty liver is related to the cause.
It is important to remember that simple fatty liver may not require treatment. The benefit of weight loss, dietary fat restriction, and exercise in obese patients is inconsistent.
Reducing or eliminating alcohol use can improve fatty liver due to alcohol toxicity. Controlling blood sugar may reduce the severity of fatty liver in patients with diabetes.



6.11 Hypercholesterolemia:

a. Hypercholesterolemia, or high cholesterol, occurs when there is too much cholesterol in the body.
b. Cholesterol is a soft, waxy, fat-like substance that is a natural component of all the cells of the body.
c. High cholesterol raises risk for heart disease, heart attack, and stroke. When there is too much cholesterol circulating in the blood, it can create sticky deposits (called plaque) along the artery walls. Plaque can eventually narrow or block the flow of blood to the brain, heart, and other organs. And blood cells that get caught on the plaque form clots, which can break loose and completely block blood flow through an artery, causing heart attack or stroke.
d. There are two types of cholesterol -- HDL (high-density lipoproteins, or "good" cholesterol) and LDL (low-density lipoproteins, or "bad" cholesterol).
e. The amount of HDL relative to LDL is considered a more important indicator of heart disease risk.
f. There is a third kind of fatty material, triglycerides, found in the blood. They also play a role (generally as triglyceride levels rise, "good" HDL cholesterol falls).
g. The usual symptoms of high cholesterol, especially in early stages. The only way to determine cholesterol is high is through a blood test.
h. **The most important risk factors for high cholesterol are:** Being overweight or obese, Eating a diet high in saturated fat and trans fatty acids (found in processed and fried foods), Not getting enough exercise, Family history of heart disease, High blood pressure, Smoking, Diabetes etc
i. **Treatment Approach:** Lowering your cholesterol level reduces your risk of heart disease and stroke. Changes in lifestyle -- better diet, more exercise and specific cholesterol-lowering medications are often prescribed like, Lovastatin, Pravastatin, Rosuvastatin, Simvastatin, Atorvastatin or Fluvastatin, etc

6.12 Total Cholesterol Levels (Mg/Dl):

a. **Desirable:** Below 200
b. **Borderline high:** 200 - 239
c. **High:** Above 240
d. Optimal for people with heart disease or who are at high risk: Below 70
e. **Optimal for people at risk of heart disease:** Below 100

f. **Optimal:** 100 - 129
g. **Borderline high:** 130 - 159
h. **High:** 160 – 189

HDL Cholesterol Level (Mg/Dl) S:

a. **Poor:** Below 40
b. **Acceptable:** 40 - 59
c. **Optimal:** 60 or above

Triglyceride Levels (Mg/Dl):

a. **Optimal:** Below 150
b. **Borderline high:** 150 - 199
c. **High:** Above 200

6.13 Chapter at a Glance:

Term	Description
LTs	Leukotrienes
TAG	Triacylglycerol
ACP	Acyl carrier protein
PGs	Prostaglandins
TXs	Thromboxanes
PGI	Prostacyclins

6.14 Exercises:

6.14.1 Multiple Choice Questions:

1. An example of a hydroxy fatty acid is

(a) Ricinoleic acid (b) Crotonic acid
(c) Butyric acid (d) Oleic acid

2. An example of a saturated fatty acid is

(a) Palmitic acid (b) Oleic acid
(c) Linoleic acid (d) Erucic acid

3. In humans, a dietary essential fatty acid is

(a) Palmitic acid (b) Stearic acid
(c) Oleic acid (d) Linoleic acid

4. Glycosphingolipids are a combination of

(a) Ceramide with one or more sugar residues
(b) Glycerol with galactose
(c) Sphingosine with galactose
(d) Sphingosine with phosphoric acid

5. The importance of phospholipids as constituent of cell membrane is because they possess

(a) Fatty acids (b) Both polar and no polar groups
(c) Glycerol (d) Phosphoric acid

6. The number of ml of N/10 KOH required to neutralize the fatty acids in the distillate from 5 gm of fat is called

(a) Reichert-Meissel number (b) Polenske number
(c) Acetyl number (d) Nonvolatile fatty acid number

7. Deterioration of food (rancidity) is due to presence of

(a) Cholesterol (b) Vitamin E
(c) Peroxidation of lipids (d) Phenolic compounds

8. Molecular formula of cholesterol is

(a) C27H45OH (b) C29H47OH
(c) C29H47OH (d) C23H41OH

9. The cholesterol molecule is

(a) Benzene derivative (b) Quinoline derivative
(c) Steroid (d) Straight chain acid

10. Salkowski test is performed to detect

(a) Glycerol (b) Cholesterol
(c) Fatty acids (d) Vitamin D

6.14.2 Short Answer Questions:

1. Give an account on Hypercholesterolemia.

2. Give an account on atherosclerosis.
3. Give an account on fatty liver.
4. Write a note on ketoacidosis.
5. Write a brief account of biological significance of cholesterol.

6.14.3 Long Answer Questions:

1. Write a note on beta oxidation of saturated fatty acids.
2. Give an account of formation of ketone bodies.
3. Give the importance of lipids in obesity and its control.
4. Give an account of disorders related to lipid metabolism.
5. Guve the detailed synthesis of steroid hormone and vitamin D from cholesterol.

Answer Key MCQS:

(1) – (a), (2) – (a), (3) – (d), (4) – (a), (5) – (a), (6) – (c), (7) – (a), (8) – (c), (9) – (c), (10) – (d).

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8. Each double bond in the fatty acid is indicated by Δx, where the double bond is located on the x^{th} carbon–carbon bond, counting from the carboxylic acid end.
9. Essential fatty-acids lubricate skin prevent pressure sores (see "suggested reading at end)
10. In n minus x (also ω−x or omega-x) nomenclature a double bond of the fatty acid is located on the xth carbon–carbon bond, counting from the terminal methyl carbon (designated as n or ω) toward the carbonyl carbon.
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Chapter 7

Amino Acid Metabolism and Related Diseases

7.1 Introduction:

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids.

Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized.

This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen.

7.2 Transamination:

7.2.1 Funneling Amino Groups to Glutamate:

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate (Figure. 7.1.), producing α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming glutamate.

Glutamate produced by transamination can be oxidatively deaminated or used as an amino group donor in the synthesis of nonessential amino acids.

This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases (also called transaminases).

These enzymes are found in the cytosol and mitochondria of cells throughout the body.

All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism.

[Note: These two amino acids lose their α-amino groups by deamination]

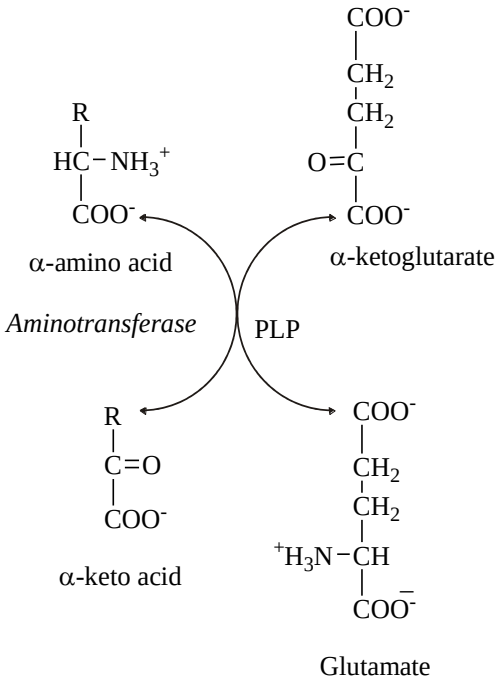

Figure 7.1: Aminotransferase Reaction using α -Ketoglutarate as the Amino Group Acceptor. PLP = Pyridoxal Phosphate:

7.3 Substrate Specificity:

Each aminotransferase is specific for one or, at most, a few amino group donors.

Aminotransferases are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as shown in Figure 7.2.

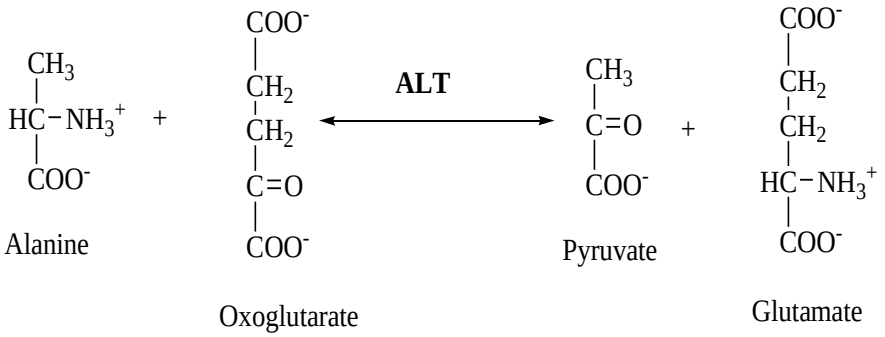

Figure 7.2: Reactions Catalyzed During Amino Acid Catabolism. A. Alanine Aminotransferase (ALT). B. Aspartate Aminotransferase (AST). PLP = Pyridoxal Phosphate.

- **Alanine Aminotransferase:** ALT is present in many tissues. The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most aminotransferases) functions in the direction of glutamate synthesis. [Note: In effect, glutamate acts as a collector of nitrogen from most amino acids.]
- **Aspartate Aminotransferase:** AST is an exception to the rule that aminotransferases funnel amino groups to form glutamate. During amino acid catabolism, AST primarily transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle. Like other transaminations, the AST reaction is reversible.

a. Mechanism: All aminotransferases require the coenzyme pyridoxal phosphate (aderivative of vitamin B_6), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme.

Aminotransferases act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme. Figure 7.2 shows these two component reactions for the transamination catalyzed by AST.

b. Equilibrium: For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

c. Diagnostic Value: Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of aminotransferases indicate damage to cells rich in these enzymes.

For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two aminotransferases, AST and ALT, are of particular diagnostic value when they are found in the plasma.

- **Hepatic Disease:** Plasma *AST* and *ALT* are elevated in nearly all hepatic diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. *ALT* is more specific than *AST* for liver disease, but the latter is more sensitive because the liver contains larger amounts of AST. Serial measurements of AST and ALT (liver function tests) are often useful in determining the course of liver damage. Figure 7.3 shows the early release of ALT into the blood, following ingestion of a liver toxin. [**Note:** The elevation in bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin.]

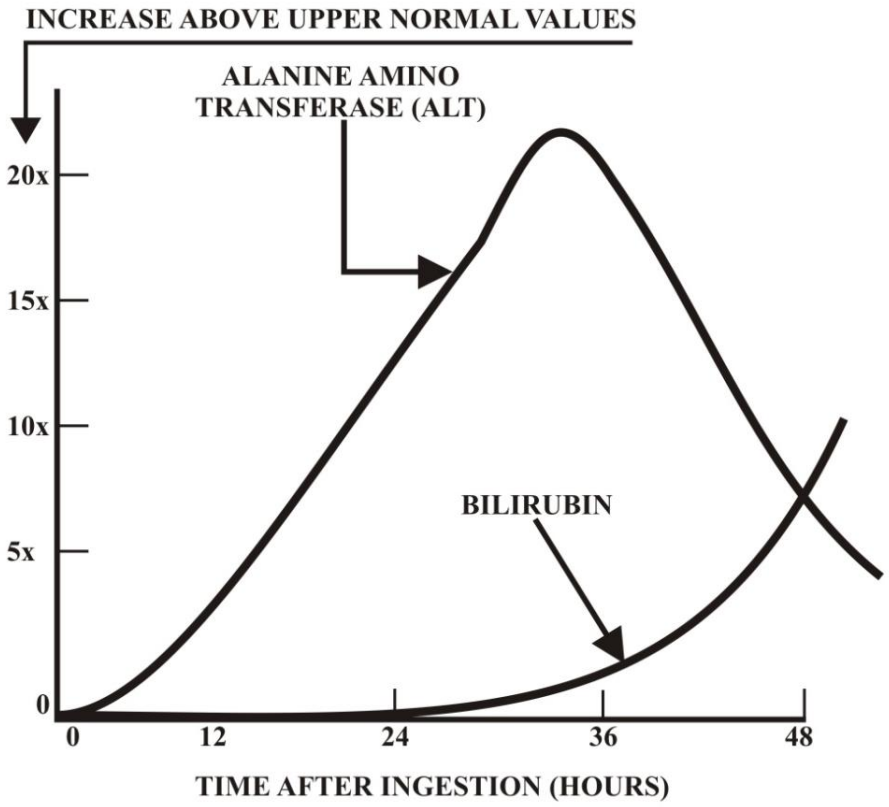

Figure 7.3: Pattern of ALT and Bilirubin in the Plasma, following Poisoning by Ingestion of the Toxic Mushroom Amanita Phalloides

- **Nonhepatic Disease:**

Aminotransferases may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle.

However, these disorders can usually be distinguished clinically from liver disease.

7.4 Oxidative Deamination: Amino Group Removal:

In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as free ammonia (Figure. 7.4). These reactions occur primarily in the liver and kidney.

They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis.

[**Note:** Ammonia exists primarily as ammonium (NH_4) in aqueous solution, but it is the unionized from (NH_3) that crosses membranes].

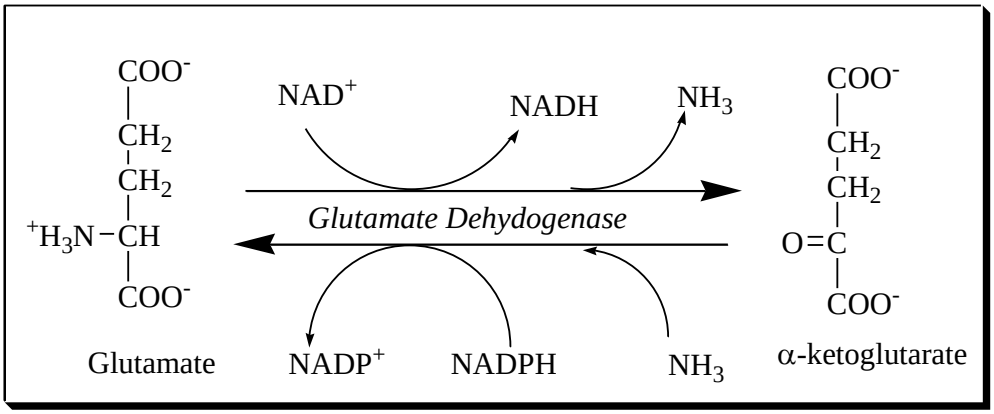

Figure 7.4: Oxidative Deamination by Glutamate Dehydrogenase

[**Note:** The Enzyme is Unusual in that It uses Both NAD^+ (Nicotinamide Adenine Dinucleotide) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate) NH_3 = Ammonia.

a. Glutamate Dehydrogenase:

As described above, the amino groups of most aminoacids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase (Figure. 7.4). Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia.

- **Coenzymes: *GDH,*** a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD^+) or its phosphorylated reduced form (NADPH) as a coenzyme. NAD^+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, whereas NADPH is used in reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton).
- **Reaction Direction:** The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia. High ammonia levels are required to drive the reaction to glutamate synthesis.
- Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of GDH, whereas adenosine diphosphate is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by GDH is high, facilitating energy production from the carbon skeletons derived from amino acids.

a. D-Amino Acid Oxidase: D-Amino acids are supplied by the diet but are not used in the synthesis of mammalian proteins. They are, however, efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by flavin adenine dinucleotide–dependent D-amino acid oxidase (DAO). The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. [**Note:** DAO degrades D-serine, the isomeric form of serine that modulates N-methyl-D-aspartate (NMDA)-type glutamate receptors. Increased DAO activity has been linked to increased susceptibility to schizophrenia. DAO also converts glycine to glyoxylate] L-Amino acid oxidases are found in snake venom.

b. Ammonia Transport to the Liver:

Two mechanisms are available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia.

The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to glutamate and ammonia. The glutamate is oxidatively deaminated to ammonia and α-ketoglutarate by GDH. The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the BCAA isoleucine and valine. Alanine is transported in the blood to the liver, where it is transaminated by ALT to pyruvate.

The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle. The glutamate product of ALT can be deaminated by GDH, generating ammonia. Thus, both alanine and glutamine carry ammonia to the liver.

7.5 Urea Cycle:

Urea is the major disposal form of amino groups derived from amino acids and accounts for ~90% of the nitrogen-containing components of urine.

One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. [**Note:** Glutamate is the immediate precursor of both ammonia (through oxidative deamination by **GDH**) and aspartate nitrogen (through transamination of oxaloacetate by **AST**).] The carbon and oxygen of urea are derived from CO_2 as (HCO_3) CO_2 (as HCO_3). Urea is produced by the liver transported in the blood to the kidneys for and then is in the urine excretion.

a. Reactions:

The first two reactions leading to the synthesis of urea occur in the mitochondrial matrix, whereas the remaining cycle enzymes are located in the cytosol. [**Note:** Gluconeogenesis and heme synthesis also involve both the mitochondrial matrix and the cytosol.]

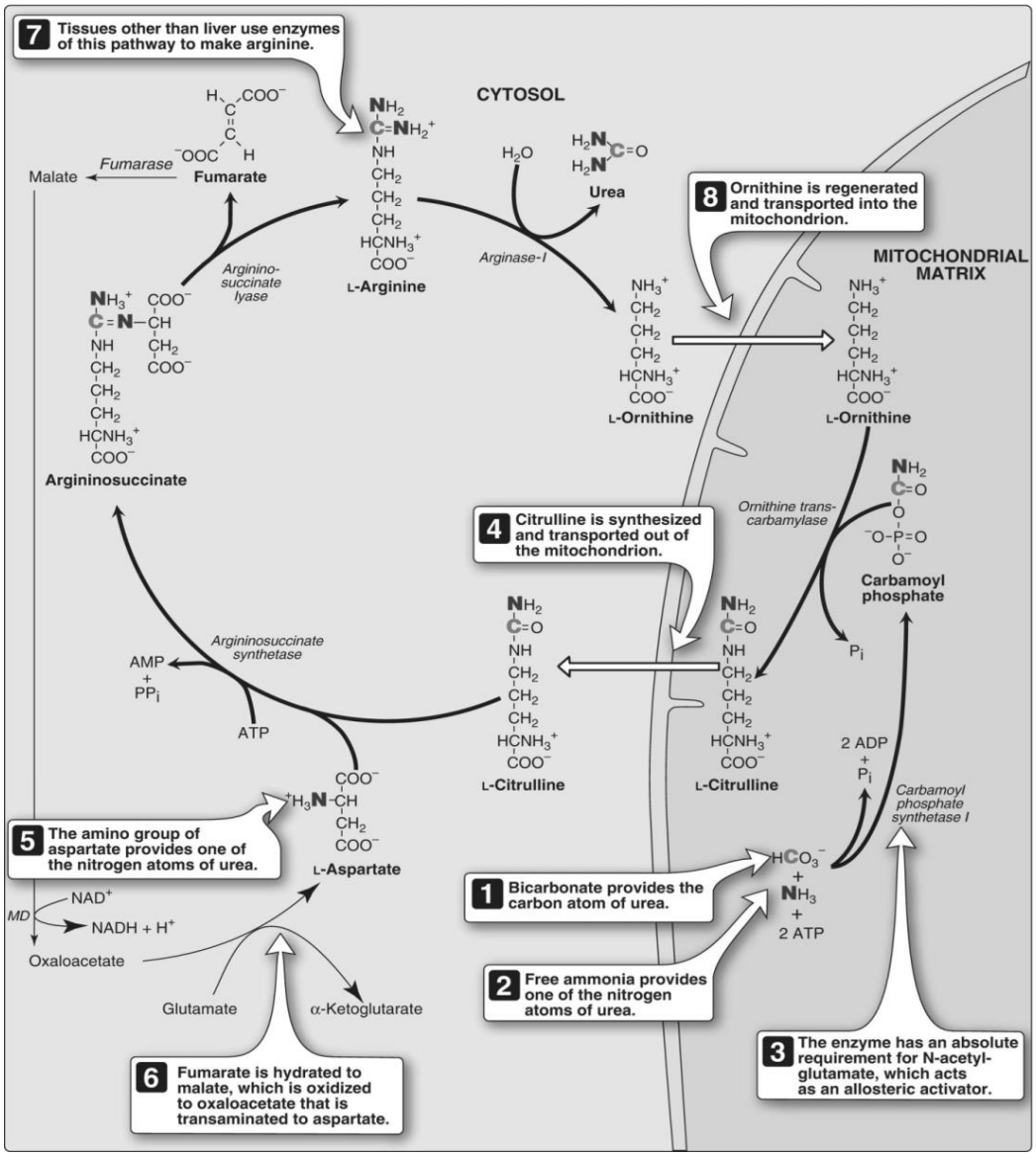

Figure 7.5: Reactions of the Urea Cycle

[**Note:** An Antiporter Transports Citrulline and Ornithine across the Inner Mitochondrial Membrane.] ADP = Adenosine Diphosphate; AMP = Adenosine Monophosphate; Ppi = Pyrophosphate; Pi = Inorganic Phosphate; NAD (H) = Nicotinamide Adenine Dinucleotide; MD = Malate Dehydrogenase.

- **Carbamoyl Phosphate Formation:** Formation of carbamoyl phosphate by carbamoyl phosphate synthetase I (CPS I) is driven by cleavage of two molecules of ATP. Ammonia incorporated into carbamoyl phosphate is provided primarily by the oxidative deamination of glutamate by mitochondrial GDH. Ultimately, the nitrogen atom derived from this ammonia becomes one of the nitrogens of urea. CPS I requires N-

acetylglutamate (NAG) as a positive allosteric activator (see Figure. 7.5). [Note: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines. It does not require NAG, uses glutamine as the nitrogen source, and occurs in the cytosol.]

- **Citrulline Formation:** The carbamoyl portion of carbamoyl phosphate is transferred to ornithine by ornithine transcarbamylase (OTC) as the phosphate is released as inorganic phosphate. The reaction product, citrulline, is transported to the cytosol. [**Note:** Ornithine and citrulline move across the inner mitochondrial membrane via an antiporter. These basic amino acids are not incorporated into cellular proteins because there are no codons for them.] Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the tricarboxylic acid (TCA) cycle.
- **Argininosuccinate Formation:** Argininosuccinate synthetase combines citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea. The formation of argininosuccinate is driven by the cleavage of ATP to adenosine monophosphate and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea.
- **Argininosuccinate Cleavage:** Argininosuccinate is cleaved by argininosuccinate lyase to yield arginine and fumarate. The arginine serves as the immediate precursor of urea. The fumarate is hydrated to malate, providing a link with several metabolic pathways. Malate can be oxidized by malate dehydrogenase to oxaloacetate, which can be transaminated to aspartate and enter the urea cycle. Alternatively, malate can be transported into mitochondria via the malate–aspartate shuttle, reenter the TCA cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis [**Note:** Malate oxidation generates NADH for oxidative phosphorylation, thereby reducing the energy cost of the urea cycle.]
- **Arginine Cleavage to Ornithine and Urea:** Arginase-I hydrolyzes arginine to ornithine and urea and is virtually exclusive to the liver. Therefore, only the liver can cleave arginine, thereby synthesizing urea, whereas other tissues, such as the kidney, can synthesize arginine from citrulline. [**Note:** Arginase-II in kidneys controls arginine availability for nitric oxide synthesis.]
- **Fate of Urea:** Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine. A portion of the urea diffuses from the blood into the intestine and is cleaved to CO_2 and ammonia by bacterial urease. The ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut.

The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the hyperammonemia often seen in these patients.

Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this ammonia production.

7.6 Amino Acids that form Fumarate:

a. Phenylalanine and Tyrosine: Hydroxylation of phenylalanine produces tyrosine (Figure. 7.6). This irreversible reaction, catalyzed by tetrahydrobiopterin-requiring phenylalanine hydroxylase (PAH), initiates the catabolism of phenylalanine. Thus, phenylalanine metabolism and tyrosine metabolism merge, leading ultimately to fumarate and acetoacetate formation. Therefore, phenylalanine and tyrosine are both glucogenic and ketogenic.

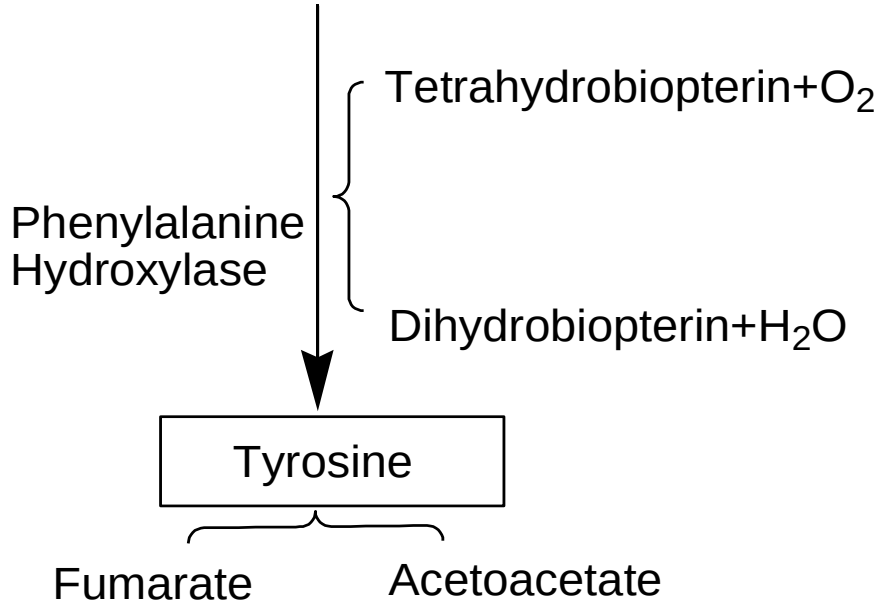

Figure 7.6: Degradation of Phenylalanine.

b. Inherited Deficiencies: Inherited deficiencies in the enzymes of phenylalanine and tyrosine metabolism lead to the diseases phenylketonuria (PKU), tyrosinemia, and alkaptonuria as well as the condition of albinism.

These single gene disorders, a subset of the inborn errors of metabolism, are caused by mutations that generally result in abnormal proteins, most often enzymes. The inherited defects may be expressed as a total loss of enzyme activity or, more frequently, as a partial deficiency in catalytic activity.

Without treatment, the amino acid disorders almost invariably result in intellectual disability or other developmental abnormalities as a consequence of harmful accumulation of metabolites.

Although >50 of these disorders have been described, many are rare, occurring in <1 per 250,000 in most populations. Collectively, however, they constitute a very significant portion of pediatric genetic diseases.

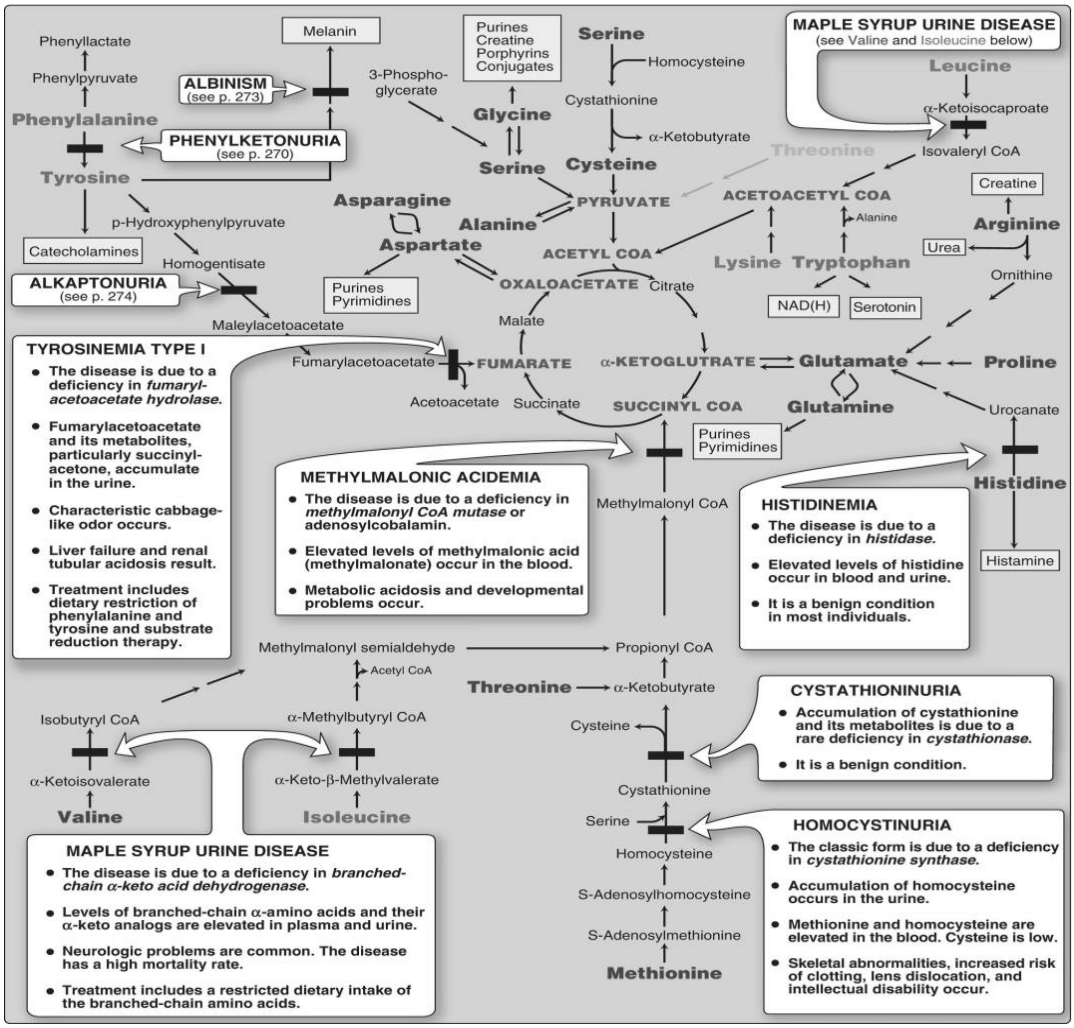

Figure 7.7: Summary of the Metabolism of Amino Acids in Humans. Genetically Determined Enzyme Deficiencies are summarized in White Boxes. Nitrogen- Containing Compounds Derived from Amino Acids are Shown in Small, Yellow Boxes. Classification of Amino Acids is Color Coded: Red = Glucogenic; Brown = Glucogenic and Ketogenic; Green = Ketogenic. Compounds in BLUE ALL CAPS Are the Seven Metabolites to which all Amino Acid Metabolism Converges. Coa = Coenzyme A; NAD (H) = Nicotinamide Adenine Dinucleotide.

7.7 Phenylketonuria:

PKU is the most common clinically encountered inborn error of amino acid metabolism (incidence 1:15,000). It is caused by a deficiency of PAH (Figure. 7.8). Biochemically, PKU is characterized by hyperphenylalaninemia. Phenylalanine is present in high concentrations (ten times normal) not only in plasma but also in urine and body tissues. Tyrosine, which normally is formed from phenylalanine by PAH, is deficient. Treatment includes dietary restriction of phenylalanine and supplementation with tyrosine.

[**Note:** Hyperphenylalaninemia may also be caused by rare deficiencies in any of the several enzymes required to synthesize BH_4 or in dihydropteridine reductase, which regenerates BH_4 from BH_2 (Figure.7.8).

Such deficiencies indirectly raise phenylalanine concentrations, because PAH requires BH4 as a coenzyme.

BH_4 is also required for tyrosine hydroxylase and tryptophan hydroxylase, which catalyze reactions leading to the synthesis of neurotransmitters, such as serotonin and the catecholamines.

Simply restricting dietary phenylalanine does not reverse the central nervous system effects due to deficiencies in neurotransmitters.

Supplementation with BH_4 and replacement therapy with L-3, 4- dihydroxyphenylalanine and 5-hydroxytryptophan (products of the affected tyrosine hydroxylase– and tryptophan hydroxylase–catalyzed reactions) improves the clinical outcome in these variant forms of hyperphenylalaninemia, although the response is unpredictable.]

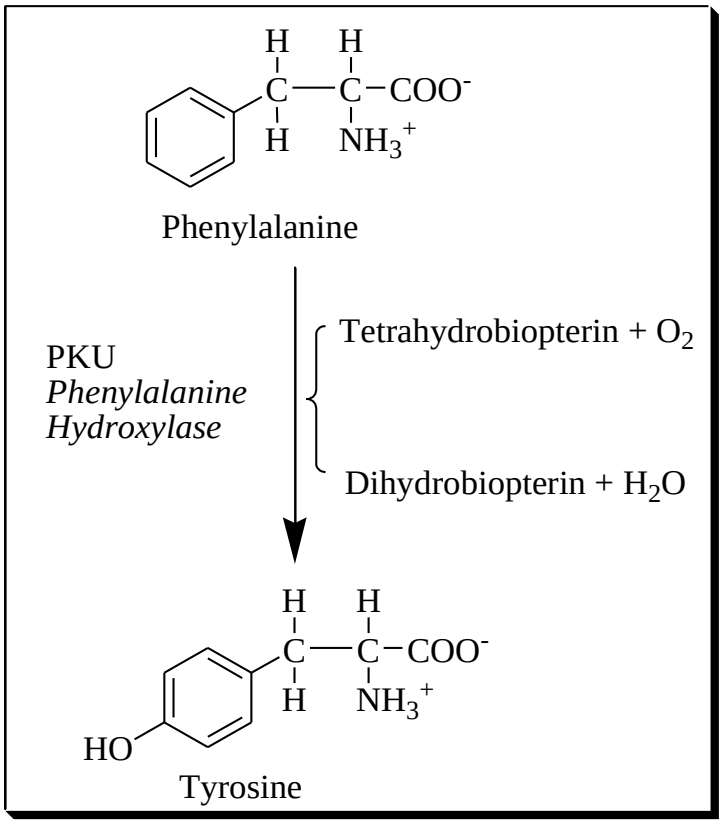

Figure 7.8: A Deficiency in Phenylalanine Hydroxylase Results in the Disease Phenylketonuria (PKU)

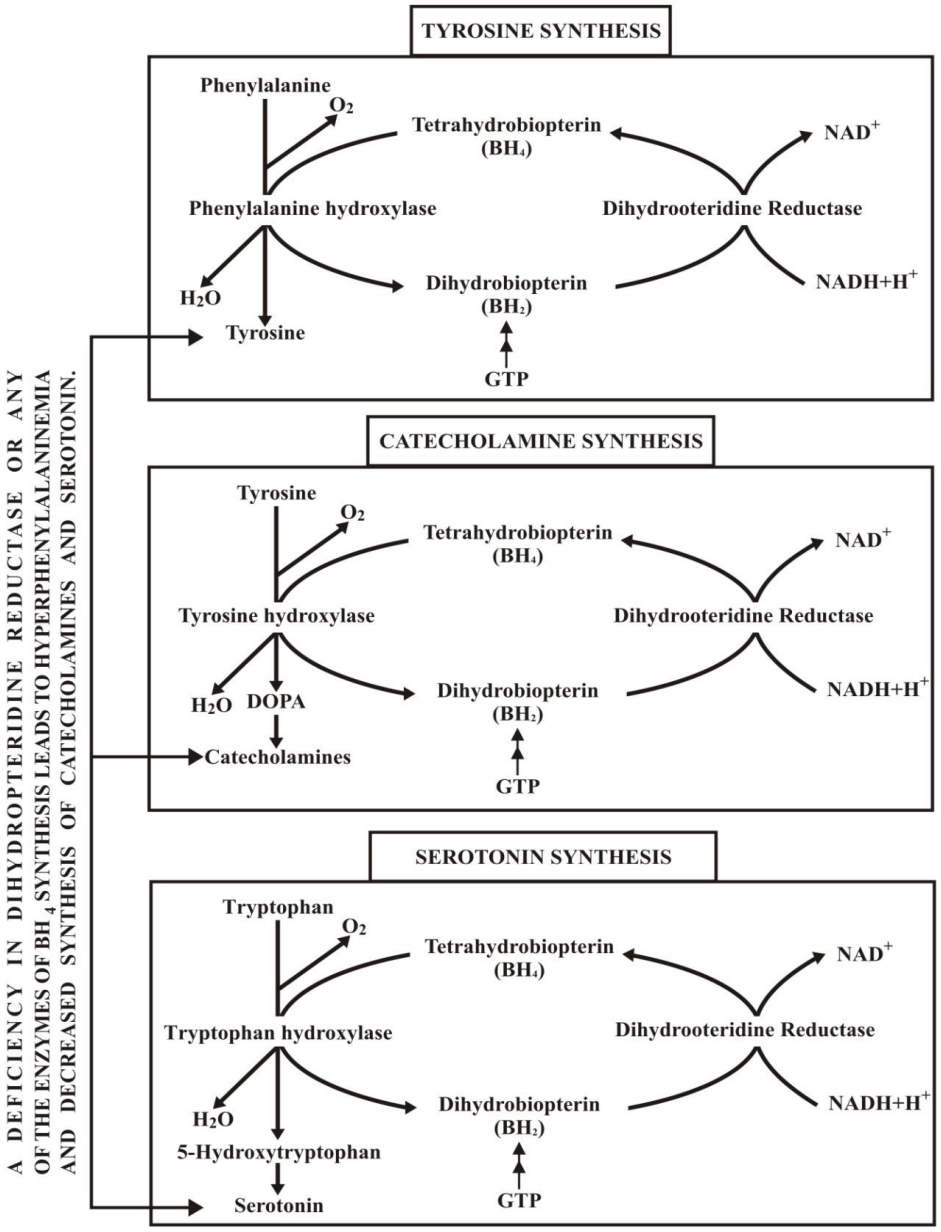

Figure 7.9: Biosynthetic Reactions Involving Amino Acids and Tetrahydrobiopterin

[**Note:** Aromatic amino acid hydroxylases use BH_4 and not PLP (pyridoxal phosphate).] NAD (H) = nicotinamide adenine dinucleotide; GTP = guanosine triphosphate; DOPA = L-3, 4-dihydroxyphenylalanine; O_2 = oxygen.

a. **Elevated Phenylalanine Metabolites:** Phenylpyruvate (a phenylketone), phenylacetate, and phenyllactate, which are not normally produced in significant amounts in the presence of functional PAH, are elevated in PKU. These metabolites give urine a characteristic musty ("mousy") odor.

b. **Central Nervous System Effects:** Severe intellectual disability, developmental delay, microcephaly, and seizures are characteristic findings in untreated PKU. The affected individual typically shows symptoms of intellectual disability by age 1 year and rarely achieves an intelligence quotient (IQ) >. [**Note:** These clinical manifestations are now rarely seen as a result of newborn screening programs, which allow early diagnosis and treatment.]

Figure 7.10: Typical Intellectual Ability in Untreated Patients of Different Ages with Phenylketonuria. IQ = Intelligence Quotient

c. **Hypopigmentation:** Patients with untreated PKU may show a deficiency of pigmentation (fair hair, light skin color, and blue eyes). The hydroxylation of tyrosine by copper-requiring tyrosinase, which is the first step in the formation of the pigment melanin, is decreased in PKU because tyrosine is decreased.

7.8 Newborn Screening and Diagnosis:

Early diagnosis of PKU is important because the disease is treatable by dietary means. Because of the lack of neonatal symptoms, laboratory testing for elevated blood levels of phenylalanine is mandatory for detection. However, the infant with PKU frequently has normal blood levels of phenylalanine at birth because the mother clears increased blood phenylalanine in her affected fetus through the placenta.

Normal levels of phenylalanine may persist until the newborn is exposed to 24–48 hours of protein feeding. Thus, screening tests are typically done after this time to avoid false negatives. For newborns with a positive screening test, diagnosis is confirmed through quantitative determination of phenylalanine levels.

7.8.1 Prenatal Diagnosis: Classic PKU is caused by any of 100 or more different mutations in the gene that encodes PAH. The frequency of any given mutation varies among populations, and the disease is often doubly heterozygous (that is, the PAH gene has a different mutation in each allele). Despite this complexity, prenatal diagnosis is possible.

a. **Treatment:** Because most natural protein contains phenylalanine, an essential amino acid, it is impossible to satisfy the body's protein requirement without exceeding the phenylalanine limit when ingesting a normal diet. Therefore, in PKU, blood phenylalanine level is maintained close to the normal range by feeding synthetic amino acid preparations free of phenylalanine, supplemented with some natural foods (such as fruits, vegetables, and certain cereals) selected for their low phenylalanine content. The amount is adjusted according to the tolerance of the individual as measured by blood phenylalanine levels.

The earlier treatment is started, the more completely neurologic damage can be prevented. Individuals who are appropriately treated can have normal intelligence. **[Note:** Treatment must begin during the first 7–10 days of life to prevent cognitive impairment.] Because phenylalanine is an essential amino acid, overzealous treatment that results in blood phenylalanine levels below normal is avoided. In patients with PKU, tyrosine cannot be synthesized from phenylalanine, and, therefore, it becomes an essential amino acid and must be supplied in the diet.

Discontinuance of the phenylalanine-restricted diet in early childhood is associated with poor performance on IQ tests. Adult PKU patients show deterioration of IQ scores after discontinuation of the diet. Therefore, lifelong restriction of dietary phenylalanine is recommended. [**Note:** Individuals with PKU are advised to avoid aspartame, an artificial sweetener that contains phenylalanine.]

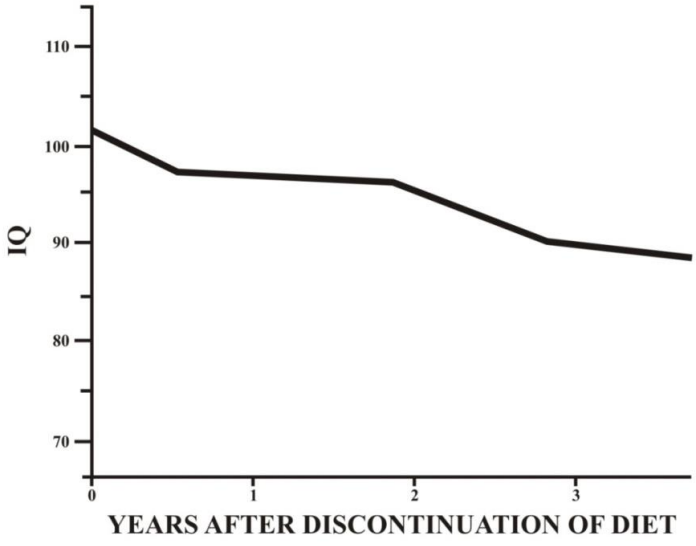

Figure 7.11: Changes in Intelligence Quotient (IQ) Scores after Discontinuation of Low-Phenylalanine Diet in Patients with Phenylketonuria

- **Maternal Phenylketonuria:** If women with PKU who are not on a low- phenylalanine diet become pregnant, the offspring can be affected with maternal PKU syndrome. High blood phenylalanine in the mother has a teratogenic effect, causing microcephaly and congenital heart abnormalities in the fetus. Because these developmental responses to

high phenylalanine occur during the first months of pregnancy, dietary control of blood phenylalanine must begin prior to conception and be maintained throughout the pregnancy.

7.8.2 Maple Syrup Urine Disease:

Maple syrup urine disease (MSUD) is a rare (1:185,000), autosomal- recessive disorder in which there is a partial or complete deficiency in BCKD, the mitochondrial enzyme complex that oxidatively decarboxylates leucine, isoleucine, and valine. These BCAA and their corresponding α-keto acids accumulate in the blood, causing a toxic effect that interferes with brain functions. The disease is characterized by feeding problems, vomiting, ketoacidosis, changes in muscle tone, neurologic problems that can result in coma (primarily because of the rise in leucine), and a characteristic maple syrup–like odor of the urine because of the rise in isoleucine. If untreated, the disease is fatal. If treatment is delayed, intellectual disability results.

a. Classification: MSUD includes a classic type and several variant forms. The classic, neonatal-onset form is the most common type of MSUD. Leukocytes or cultured skin fibroblasts from these patients show little or no BCKD activity. Infants with classic MSUD show symptoms within the first several days of life. If not diagnosed and treated, classic MSUD is lethal in the first weeks of life. Patients with intermediate forms have a higher level of enzyme activity (up to 30% of normal). The symptoms are milder and show an onset from infancy to adolescence. Patients with the rare thiamine-dependent variant of MSUD respond to large doses of this vitamin.

b. Screening and Diagnosis: As with PKU, prenatal diagnosis and newborn screening are available and most affected individuals are compound heterozygotes.

c. Treatment: MSUD is treated with a synthetic formula that is free of BCAA, supplemented with limited amounts of leucine, isoleucine, and

- **Homocystinuria**

The homocystinurias are a group of disorders involving defects in the metabolism of Hcy. These autosomal-recessive diseases are characterized by high urinary levels of Hcy, high plasma levels of Hcy and methionine, and low plasma levels of cysteine. The most common cause of homocystinuria is a defect in the enzyme cystathionine β-synthase, which converts Hcy to cystathionine. Individuals homozygous for cystathionine β-synthase deficiency exhibit dislocation of the lens (ectopia lentis), skeletal anomalies (long limbs and fingers), intellectual disability, and an increased risk for developing thrombi (blood clots). Thrombosis is the major cause of early death in these individuals. Treatment includes restriction of methionine and supplementation with vitamin B_{12} and folate. Additionally, some patients are responsive to oral administration of pyridoxine (vitamin B_6), which is converted to pyridoxal phosphate, the coenzyme of cystathionine β-synthase. These patients usually have a milder and later onset of clinical symptoms compared with B6-nonresponsive patients. [**Note:** Deficiencies in methylcobalamin or N^5, N10-MTHF reductase ([MTHFR]; also result in elevated Hcy.]

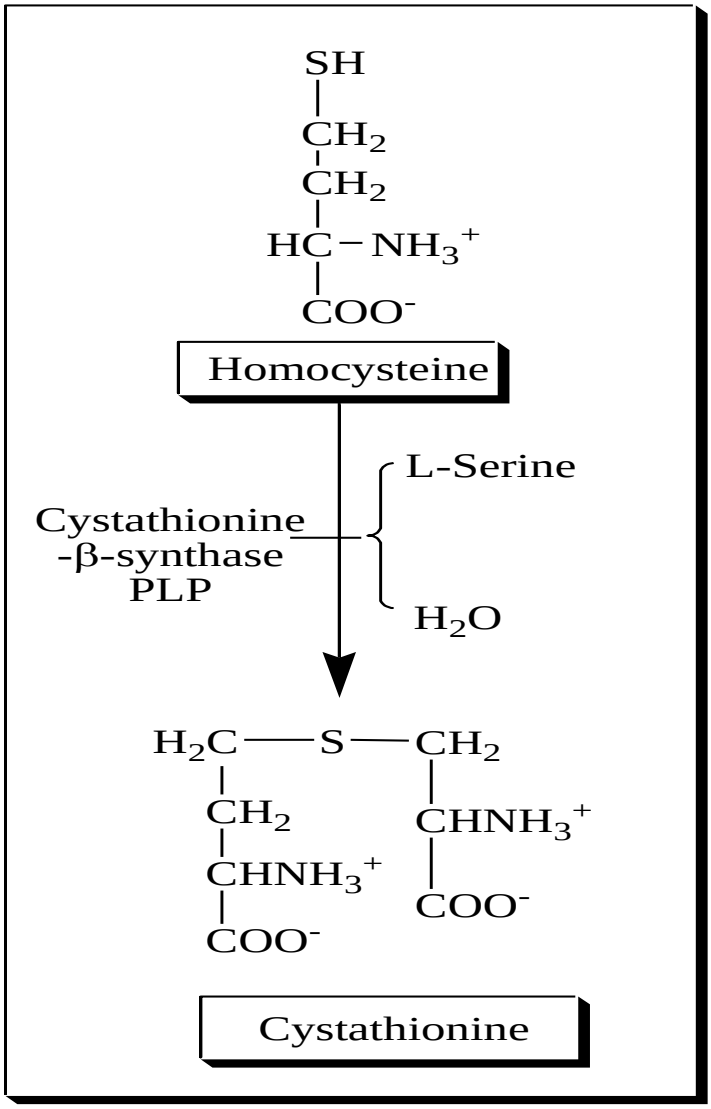

Figure 7.12: Enzyme Deficiency in Homocystinuria. PLP = Pyridoxal Phosphate.

7.9 Alkaptonuria:

Alkaptonuria is a rare organic aciduria involving a deficiency in homogentisic acid oxidase, resulting in the accumulation of homogentisic acid (HA), an intermediate in the degradative pathway of tyrosine. The condition has three characteristic symptoms: homogentisic aciduria (the urine contains elevated levels of HA, which is oxidized to a dark pigment on standing, early onset of arthritis in the large joints, and deposition of black pigment (ochronosis) in cartilage and collagenous tissue.

Dark staining of diapers can indicate the disease in infants, but usually no symptoms are present until about age 40 years. Treatment includes dietary restriction of phenylalanine and tyrosine to reduce HA levels. Although alkaptonuria is not life threatening, the associated

arthritis may be severely crippling. [**Note:** Deficiencies in fumarylacetoacetate hydrolase, the terminal enzyme of tyrosine metabolism, result in tyrosinemia type and a characteristic cabbage-like odor to urine.] Valine to allow for normal growth and development without producing toxic levels. [Note: Elevated leucine is the cause of the neurologic damage in MSUD, and its level is carefully monitored.] Early diagnosis and lifelong dietary treatment are essential if the child with MSUD is to develop normally. [**Note:** BCAA are an important energy source in times of metabolic need, and individuals with MSUD are at risk of decompensation during periods of increased protein catabolism.]

7.10 Albinism:

Albinism refers to a group of conditions in which a defect in tyrosine metabolism results in a deficiency in the production of melanin. These defects result in the partial or full absence of pigment from the skin, hair, and eyes. Albinism appears in different forms, and it may be inherited by one of several modes: autosomal recessive (primary mode), autosomal dominant, or X linked.

Total absence of pigment from the hair, eyes, and skin, tyrosinase-negative oculocutaneous albinism (type 1 albinism), results from an absent or defective copper-requiring tyrosinase. It is the most severe form of the condition. In addition to hypopigmentation, affected individuals have vision defects and photophobia (sunlight hurts their eyes). They are at increased risk for skin cancer.

7.11 Chapter at a Glance:

Term	Description
PLP	Pyridoxal phosphate.
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
NAD+	Nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
PKU	Phenylketonuria

7.12 Exercises:

7.12.1 Multiple Choice Questions:

1. Proteins contain

(a) Only L- α - amino acids (b) Only D-amino acids
(c) DL-Amino acids (d) Both (A) and (B)

2. The optically inactive amino acid is

(a) Glycine (b) Serine
(c) Threonine (d) Valine

3. At neutral pH, a mixture of amino acids in solution would be predominantly:

(a) Dipolar ions (b) Nonpolar molecules
(c) Positive and monovalent (d) Hydrophobic

4. The true statement about solutions of amino acids at physiological pH is

(a) All amino acids contain both positive and negative charges
(b) All amino acids contain positively charged side chains
(c) Some amino acids contain only positive charge
(d) All amino acids contain negatively charged side chains

5. pH (isoelectric pH) of alanine is

(a) 6.02 (b) 6.6
(c) 6.8 (d) 7.2

6. Sulphur containing amino acid is

(a) Methionine (b) Leucine
(c) Valine (d) Asparagine

7. An example of sulphur containing amino acid is

(a) 2-Amino-3-mercaptopropanoic acid
(b) 2-Amino-3-methylbutanoic acid
(c) 2-Amino-3-hydroxypropanoic acid
(d) Amino acetic acid

8. All the following are sulphur containing amino acids found in proteins except

(a) Cysteine (b) Cystine
(c) Methionine (d) Threonine

9. An aromatic amino acid is

(a) Lysine (b) Tyrosine
(c) Taurine (d) Arginine

10. The functions of plasma albumin are

(a)	Osmosis	(b)	Transport
(c)	Immunity	(d)	both (A) and (B)

7.12.2 Short Answer Questions:

1. Define essential and non-essential amino acids.
2. Write a note on Phenylketonuria.
3. Write a note on albinism.
4. Give the Synthesis and significance of 5-HT.
5. Give the Synthesis and significance of adrenaline.

7.12.3 Long Answer Questions:

1. Describe in detail the urea cycle.
2. Write a note on hyperbilurubenia and jaundice.
3. What is deamination? Discuss the disorder related to metabolism of phenyl alanine.
4. Write a note on important neurotransmitters of sympathetic nervous system.
5. Write a note on alkeptonuria, tyrosinemia.

Answer Key MCQs:

(1) – (a), (2) – (a), (3) – (a), (4) – (a), (5) – (a), (6) – (a), (7) – (a), (8) – (d), (9) – (b), (10) – (a)

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- *He has received Eminent Scientist of the Year Award in 2018 in conference EIAEASSD at SHUATs Allahabad.*
- *He supervised 07 M. Pharm. students and 02 as under joint research project till date.*
- *He is supervising 01 Ph.D. student currently.*
- *His students received currently ICMR-SRF project for novel leads in PTP-1B inhibitors in 2018.*

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