



TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

LECTURE NOTES ON

UNIT – 2

INTERMEDIARY METABOLISM

(VCI MSVE syllabus 2016)

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2019 – 2020

INTERMEDIARY METABOLISM

ENZYMOLOGY:

- The word Enzyme (Greek; en – in; enzyme –yeast) was coined by F.T Kuhne.
- Enzymes are regarded as biological catalysts that are mostly protein molecules synthesized by the living cells, with the exception of 'Ribozymes', which are RNAs that catalyze the biochemical reactions.
- Enzymes lower the energy of activation.
- Enzymes do not alter the equilibrium of the reaction. They enhance the rate of attaining the equilibrium
- Enzymes are globular & thermolabile in character and are specific in their action.
- The molecule on which the enzyme acts to form product is known as "substrate".
- Active site is the region of the enzyme that binds to the substrate.
- Three distinct features characterize the enzymes:
 - Catalytic power
 - Specificity
 - Regulation
- A number of factors like pH, temperature, substrate concentration, presence of cofactors, inhibitors and activators can affect the rate of an enzyme-catalyzed reaction.

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

NOMENCLATURE OF ENZYMES

Trivial system of nomenclature

- Many enzymes are named by adding the suffix – “ase” to the name of the substrate on which they act on or to the reaction that they catalyze. Eg: Urease has urea as its substrate.
- However, few enzymes such as trypsin, chymotrypsin gives no hint of the substrates on which they act. Hence, a systematic nomenclature is adopted by the International Union of Biochemistry and Molecular Biology (IUBMB).

Systematic nomenclature

- The Enzyme Commission (EC) has adopted a number system in which each enzyme is given four numbers separated by dots.
- The first number is one of the six major classes.
- The second number denotes its subclass, indicating the kind of group acted on.
- The third number designates its sub- subclass, showing the type of acceptor.
- The fourth number is the enzyme's, arbitrarily assigned serial number in its sub-sub class.
- For example, EC 2.7.1.1 denotes
 - Class 2 (a transferase).
 - Subclass 7 (transfer of phosphate).
 - Sub sub class 1 (an alcohol to accept the phosphate group).
 - The final digit, 1, denotes hexokinase or ATP: D-Hexose-6 phosphate transferase, an enzyme catalyzing the phosphate transfer from ATP to the hydroxyl group on carbon 6 of glucose.

CLASSIFICATION OF ENZYMES

There are six main classes of enzymes in the order as described below:

Oxidoreductases (E.C. 1)

- Oxidoreductases catalyze oxidation- reduction reactions.
- Most of the enzymes use co- enzymes such as NAD⁺, NADP⁺ or FAD. These enzymes are sub-classed as dehydrogenases, oxidases, hydroperoxidases, and oxygenases.
- Eg: Alcohol dehydrogenase, Cytochrome oxidase

Transferases (E.C. 2)

- They catalyze group transfer reactions. To carry out the work, the enzymes require the presence of coenzymes.
- In-group transfer reactions, a portion of the substrate molecule usually binds covalently to the enzyme or its coenzyme. This group includes the kinases, transaminases, acyl transferases and transcarboxylases.
- Eg: Hexokinase, Transaminases, Methylases

Hydrolases (E.C. 3)

- They catalyze hydrolysis, by addition of water molecule across the bond they split.
- Common trivial names include esterases, peptidases, amylases and phosphatases.
- Eg: Urease, Acetylcholinesterase, Trypsin, Maltase, Lipases, Deaminases

Lyases (E.C. 4)

- They catalyze non-hydrolytic and non- oxidative elimination reactions, or lysis of a substrate, generating a double bond.
- In the reverse direction, lyases catalyze addition of one substrate to a double bond.
- Common trivial names include dehydratases and decarboxylases.
- Eg: Aldolase, Fumarase

Isomerases (E.C. 5)

- They catalyze structural change within one molecule to yield isomeric forms.
- Trivial names include epimerases, racemases and mutases.
- Eg: Glucose-6-phosphate isomerase, Alanine racemase, Glucose -1-phosphate mutase.

Ligases (E.C. 6)

- They catalyze ligation or joining of two substrates coupled to cleavage of a high-energy bond, such as ATP. Trivial names include thiokinase and carboxylase.
- Ligases are usually referred to as Synthetases – require ATP.
- Synthases- requires energy, but do not use ATP as a source.
- Eg: Acetyl Co carboxylase, DNA Ligase, Glutamine synthetase

COENZYMES, ISOENZYMES AND ALLOSTERIC ENZYMES

Co - factors and Co - enzymes

- Some enzymes require no additional chemical groups but others require an additional chemical component called cofactor for their activity.
- The cofactor may be either one or more inorganic ions such as, Fe^{2+} , Mg^{2+} and Zn^{2+} or a complex organic or metallo organic molecule called a coenzyme.
- Some enzymes require both a coenzyme and one or more metal ions for their activity. A coenzyme or metal ion that is covalently bound to the enzyme part is called as "Prosthetic group".
- The protein part of such an enzyme is called "apoenzyme" or "apoprotein".
- A completely, catalytically active enzyme together with its coenzyme and / or metal ions is called a "holoenzyme" (apoenzyme + coenzyme).

Co- enzymes

- Co –enzymes are heat stable low molecular weight organic molecules. Along with enzymes, they take part in the biochemical reactions.
- They can be easily removed from the apoenzyme.
- Coenzymes are regarded as second substrates, because, the chemical changes in the coenzymes exactly counterbalance those taking place in the substrate.
- Most of the coenzymes are synthesized from vitamins especially from B complex vitamins.
- Co- enzymes are classified into two types according to the group they transfer. Viz.,
 - *For transfer of hydrogen group*
 - NAD⁺, NADP⁺, FMN, FAD, lipoic acid, coenzyme Q.
 - *For transfer of group other than hydrogen group*
 - CoASH, thiamine pyrophosphate, pyridoxal phosphate, folate coenzyme, biotin, cobalamide (B₁₂) coenzyme, lipoic acid.
 - Many coenzymes contain adenine, ribose and phosphate or derivatives of adenosine monophosphate (AMP). Examples include NAD⁺, NADP⁺.

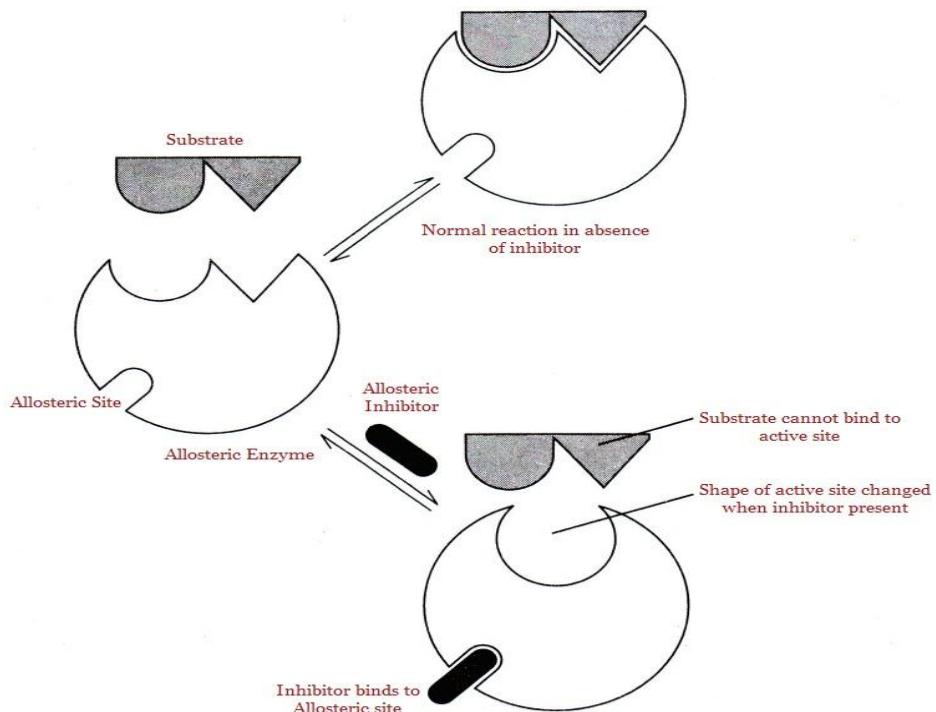
ISO ENZYMES

- Isoenzymes (isozymes) are enzymes that catalyze the same reaction, but differ in their physical properties. They have different amino acid sequence and also different properties like charge, molecular weight; hence, they can be separated by electrophoresis. Different tissues may contain different isoenzymes and these isoenzymes may differ in their affinity for substrates and they may be inhibited by different concentrations of inhibitors.
- Many isoenzymes contain different subunits in various combinations.
- One of the first enzymes found to have such multiple forms is lactate dehydrogenase (LDH) which catalyzes the reversible reaction -

- Pyruvate + NADH+H⁺ \xrightleftharpoons{LDH} Lactate + NAD⁺**
- There are five different forms present in different proportions in different tissues. The enzyme is made with two types of subunits: M (muscle) and H (heart). The different forms are H4, H3M, H2M2, HM3 and M4 (LDH 1-5 respectively). They are used to detect and differentiate the disease conditions. LDH 1& 2 are important in the diagnosis of myocardial infarctions. LDH 4 & 5 are in the diagnosis of liver disorders.

ALLOSTERIC ENZYME

- Allosteric means other than the active site. Allosteric enzyme will have a site other than the active site, where regulatory molecules called allosteric effectors can bind to enzyme and alter the enzyme activity. Allosteric molecule can increase (positive modulator) or decrease the rate of an enzyme catalyzed reaction (negative modulator).
- These enzymes are generally seen at commitment step in the metabolic pathways.
- These enzymes will have multiple subunits and are generally inhibited by feed back inhibition. i.e., inhibition of the enzyme activity by the end product of the pathway. The attachment of the molecule may be through reversible noncovalent binding.
- When reaction velocity is plotted against substrate concentration, these enzymes generally do not exhibit Michaelis-Menten behavior (hyperbolic curve), but show a sigmoidal type of curve.
- In the conversion of threonine to isoleucine, the end product isoleucine inhibits the first enzyme of the pathway, threonine dehydratase by feed back mechanism.



FACTORS AFFECTING ENZYMATIC REACTIONS

pH

- As majority of the enzymes are proteins, the activities of the enzymes are highly sensitive to a change in the pH.
- Enzymes have their own optimum pH, at which their activity is maximum. At higher or lower pH, the activity decreases.

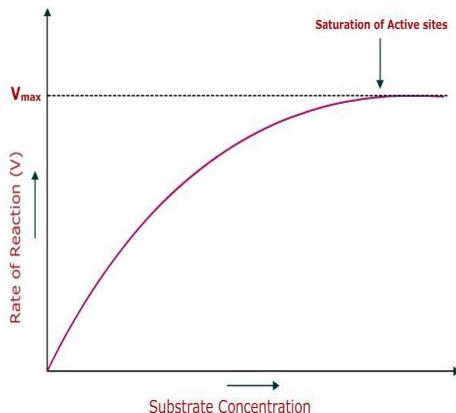
- Most of the enzymes will act within a narrow pH range of 5 – 9.
- This is a result of the effect of pH on a combination of factors like,
 - Binding of substrate to enzyme.
 - Catalytic activity of the enzyme
 - The ionization of substrate and
 - The variation of enzyme structure.
- Extremes of pH can lead to denaturation of enzymes. The initial rate for many enzymatic reactions exhibit a bell – shaped curve, when it is plotted against pH variation.
- For example, pepsin, a digestive enzyme in stomach is maximally active at pH 2. Whereas, other enzymes working at neutral pH, are denatured by the acidic environment of the stomach.

TEMPERATURE

- Most of the enzymes have an optimum temperature around 37 °C for their maximum activity.
- The reaction velocity increases with the temperature, until a peak velocity is reached (37 °C).
- This increase is due to increased number of molecules having sufficient energy to pass over the energy barrier to form the product.
- Further elevation of the temperature results in a decrease in reaction velocity due to the denaturation of the enzymes.

SUBSTRATE CONCENTRATION

- The rate or velocity of a reaction is the number of substrate molecules converted into products per unit time.
- At very low substrate concentrations, there are plenty of enzymes to interact with the substrates through the active sites. Thus, the reaction rate is proportional to the substrate concentration.
- At higher substrate concentration, all the active sites of the enzymes are saturated and attain a maximum reaction rate. Hence, further increase in substrate concentration has no or little effect on the reaction rate.
- Most of the enzymes show hyperbolic curve when reaction velocity is plotted against substrate concentration.



ENZYME CONCENTRATION

- The velocity of the enzyme catalyzed reaction increases with the increase in the concentration of the enzyme, provided, the substrate concentration is very large.

ENZYME UNITS, SPECIFIC ACTIVITY AND TURN OVER NUMBER

Enzyme units

- Enzyme activity is usually expressed quantitatively for all biochemical reactions and not the enzyme concentration (because concentration of an individual protein enzyme will be very very small when compared to the total protein concentration).
- Units of enzyme activity have been expressed in many ways.
- The International Union of Biochemistry has defined that one unit (U) of enzyme activity is the amount of enzyme that catalyzes the conversion of 1.0 micromole of substrate to product per minute under defined temperature, pH and other conditions.
- The SI unit of enzyme activity is the katal (kat).
- One katal is the transformation of 1.0 mole of substrate to product per second.
 - $1 \text{ U} = 1 \mu \text{ mol / min}$
 - $1 \text{ katal} = 1 \text{ mol / sec}$
 - $1 \text{ U} = \mu \text{ kat} / 60 = 16.67 \text{ nkat}$.

Specific activity

- Specific activity of an enzyme is defined as the number of enzyme units per milligrams of protein (U/mg of protein).
- The specific activity in SI units is given as katals per kilograms of protein.
- Specific activity is a measure of enzyme purity. It increases during purification of enzyme and becomes maximal and constant when the enzyme is in its purest state.

Turnover number

- Turnover number compares the relative activities of pure enzyme, which is the number of molecules of the substrate converted to product per enzyme molecule in a given unit of time.
- The turnover number is also called “*catalytic constant*” or “*molecular activity*”.
- For example, the constants for catalase and alpha-amylase are 5×10^6 and 1.9×10^4 respectively, indicating that catalase is about 2500 times more active than amylase.

ENZYME KINETICS AND ENZYME INHIBITION

Enzyme kinetics

- It is the study of the rate of change of substrate to form product. Velocity is the change in the concentration of substrate or product per unit time.
- Reaction velocity is referred to the initial velocity obtained immediately after a substrate is added to the enzyme. Initial rate is chosen as a measure of enzyme activity because the rate of a reaction begins to slow down as substrate becomes depleted and product builds up.

Reaction order

- If the enzyme is saturated with substrate so that no more will bind, the reaction is said to be zero order.
- If the enzyme is not saturated and the reaction is directly proportional to the substrate added, then the reaction is said to be first order.

ENZYME - SUBSTRATE COMPLEX FORMATION

- Enzyme – catalyzed reactions occur in two phases. First, substrate and enzyme interact to form an enzyme- substrate complex (rate constant k1). This complex then dissociates to yield the free enzyme plus product (rate constant k2) or it can break down to enzyme and the substrate (rate constant k3).



- Where,

E is the enzyme

S is the substrate

ES is the enzyme – substrate complex

P is the product

k1, k2, and k3 are rate constants

- Michaelis – Menten proposed a simple mechanism for enzyme-catalyzed reactions. This explains how reaction velocity varies with substrate concentration. Let us consider a simple enzymatic reaction, the conversion of substrate to product.

- In this model, the enzyme reversibly binds with the substrate forming, Enzyme – Substrate complex, which subsequently dissociates to form either free enzyme and substrate, or free enzyme and product.
- In this model, when the velocity is plotted against the increasing substrate concentration, it gives a rectangular hyperbolic curve.

MICHAELIS MENTEN EQUATION

- This is a mathematical relationship between initial velocity (V_o), maximum velocity (V_{max}) and the substrate concentration $[S]$.

$$V_o = \frac{V_{max} [S]}{K_m + [S]}$$

- Where,

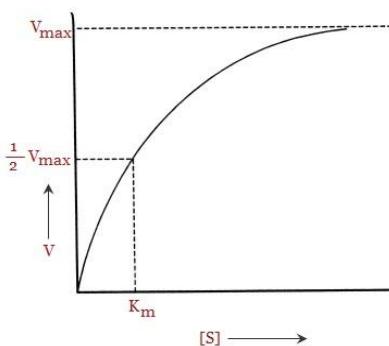
V_o is the initial velocity.

V_{max} is the maximum velocity achieved when the enzyme becomes saturated with substrate (zero order).

K_m is the Michaelis constant = $(k_1 + k_2) / k_1$.

$[S]$ is the substrate concentration.

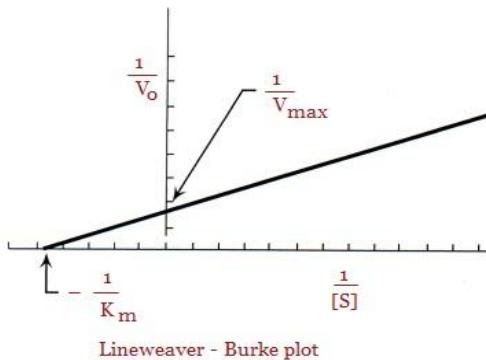
- Three useful concepts have been derived from Michaelis – Menten theory:
 - Michaelis constant (K_m), maximal velocity (V_{max}) and the Lineweaver- Burke plot.
 - K_m is the substrate concentration required to produce a reaction rate equal to half the V_{max} . K_m measures the affinity of the enzyme for the substrate. A low K_m indicates high affinity and vice versa.
 - K_m does not vary with the enzyme concentration.
 - V_{max} is the maximal velocity achieved when the enzyme is fully saturated with the substrate.



LINeweaver - BURKE PLOT (DOUBLE RECIPROCAL PLOT)

- When velocity V is plotted against [S] using Michaelis - Menten equation, it gives a hyperbolic curve.
- Using this curve, it is very difficult to determine Km and Vmax values. Hence, the Michaelis – Menten equation is rearranged into the form of a straight line to determine Km and Vmax values. This plot is known as Lineweaver – Burke plot.

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$



REVERSIBLE ENZYME INHIBITION

Enzyme inhibition

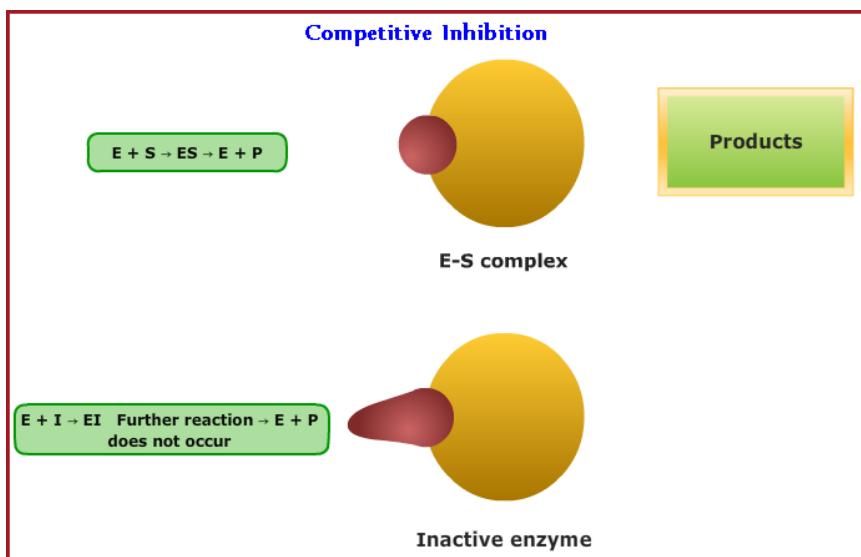
- It is the process that decreases the velocity of the enzyme-catalyzed reaction. An inhibitor of an enzyme is a compound that decreases the velocity of the enzyme-catalyzed reaction by binding to the enzyme.
- Enzyme inhibitions can be classified as reversible or irreversible. Another special type is allosteric inhibition and it is discussed under the topic 'Allosteric enzymes'

Reversible inhibition

- In reversible inhibition, an inhibitor binds reversibly to the active site or to another location on the enzyme in a way that changes the shape of the active site.
- Reversible inhibition is very important for the regulation of enzyme activities in the cell.
- Reversible inhibition can be further classified into 3 types, viz.,

- **Competitive inhibition**

- A competitive inhibitor competes with the substrate for binding to the active site of the enzyme. The structure of the inhibitor resembles the structure of the substrate.
- The degree of inhibition depends on the ratio of the concentration of inhibitor to substrate. Increasing the concentration of the substrate can reverse the inhibition.
- The K_m is increased but V_{max} remains the same.
- *Example:* Succinate dehydrogenase. This enzyme catalyzes the removal of two hydrogen atoms from succinate to form fumarate in the presence of FAD as a coenzyme. Malonate has a structure similar to succinate. Malonate has one less $-CH_2$ group than succinate. In the presence of malonate, the active site of succinate dehydrogenase reversibly binds with malonate and thereby preventing the binding of succinate.



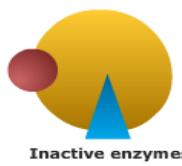
- **Non - Competitive inhibition**

- The inhibitor binds both to the free enzyme and the ES complex. The inhibitor does not compete with the substrate for binding to the active site of the enzyme.
- It reversibly binds to the enzyme at the locations other than the active site.
- The binding of the inhibitor causes a structural change in the enzyme, which damages the shape of the active site and thereby reduces the binding affinity of the substrate for the active site. The inhibitor has no structural resemblance with the substrate.
- Increasing the concentration of the substrate does not increase the rate of the enzyme reaction. In one-substrate reactions, examples of non- competitive inhibition are less common than those of competitive inhibition. In the case of Fructose bisphosphatase, AMP acts as a non-competitive inhibitor, with respect to the substrate fructose-1,6-bisphosphate.
- The K_m is not altered but the V_{max} is decreased.

Non-Competitive Inhibition



Products



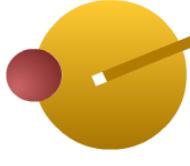
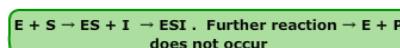
- **Un - Competitive inhibition**

- The inhibitor binds only to the ES complex.
- This type of inhibition occurs mainly in multi-substrate reactions.
- After binding of the first substrate, there is a conformational change in the enzyme, which opens the second binding site for either the co -substrate or the inhibitor.
- An example is the inhibition of alkaline phosphatase from rat intestine by L-phenylalanine.
- Both Km and Vmax are decreased.

Un-Competitive Inhibition



Products



IRREVERSIBLE ENZYME INHIBITION

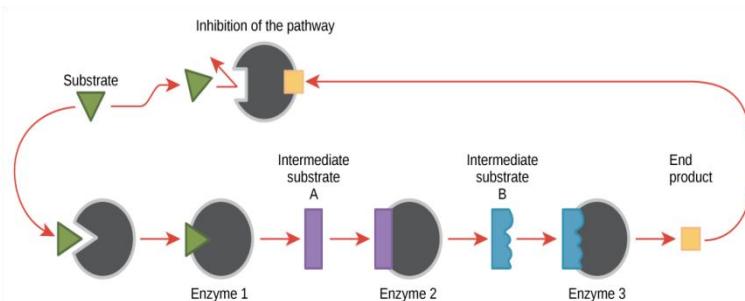
- An irreversible enzyme inhibitor reacts with an amino acid residue in the enzyme's active site and forms a stable covalent bond (complex). As a result, the enzymes are permanently inactivated.
- Irreversible inhibition of enzymes does not occur normally in the cells but follows when poison is taken from the environment.
- E.g. The reaction of aspirin, with cyclooxygenase represents one example of the action of an irreversible enzyme inhibitor. Cyclooxygenase catalyzes the first reaction in the synthesis of prostaglandins from arachidonic acid; aspirin, acetylates the amino acid serine present in the active site. This chemical modification is stable and leads to irreversible inhibition.

Suicidal inhibitor

- It is a type of irreversible enzyme inhibitor.
- These inhibitors are not reactive before binding to the active site of the enzyme. Only after interaction with the enzyme, the inhibitors are converted into an active form, which becomes tightly bound with the enzyme, and thereby inactivating the enzyme.
- E.g. Allopurinol, a drug used to treat gout, is very effective through suicide inhibition of xanthine oxidase

Feedback inhibition

- Feedback inhibition is defined as the process in which the end product of a reaction inhibits or controls the action of the enzyme that helped produce it.
- Feedback inhibition acts at the first committed step of the pathway mostly.
- The pathway steps regulated by feedback inhibition are often catalyzed by allosteric enzymes.
- In the conversion of threonine to isoleucine, the end product isoleucine inhibits the first enzyme of the pathway, threonine dehydratase by feed back mechanism.



BIOENERGETICS AND BIOLOGICAL OXIDATION

THERMODYNAMICS

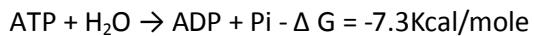
- Thermodynamics is the study of the laws that govern the conversion of energy from one form to another form, the direction of heat flow from one system to another and the availability of energy to do the work.
- Free energy changes in biological reactions are governed by first and second laws of thermodynamics.
- The first law states that total energy of the universe is constant. The second law states that universe will tend to be more disordered. In all natural processes, the entropy of the universe increases.

BIOENERGETICS

- It is the study of energy change that occurs during a biochemical reaction.
- A biochemical reaction involves rearrangement of atoms.

System

- In a reaction mixture, the reactants and products are considered as system. All the rest surrounding the system is called surrounding, which includes the reaction vessel, the outside room. The system + surrounding are known as universe.
- Every system has a definite amount of energy. A system can lose energy to the surrounding, or gain energy from the surrounding.
- The direction in which a reaction proceeds is indicated by the free energy change. It is the energy stored within the structure of the molecule, which is released during the course of a reaction. It is not possible to measure the free energy content of a substance. But when a substance, say 'S' is converted to the product 'P', it is possible to measure the change in free energy. This is the maximum amount of energy released when 'S' is converted to 'P'.
- When the free energy content of the product P is less than S the ΔG will be negative (loss of free energy). The reaction is called exergonic and proceeds spontaneously.

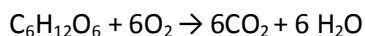


- The $+ \Delta G$ indicates that energy must be supplied to the reactants to form the product. This reaction cannot proceed spontaneously and is known as endergonic reaction. It is the conversion of P to S.



- In general, the catabolic reactions (break-down) are exergonic and the anabolic reactions (synthetic reactions) are endergonic in nature.

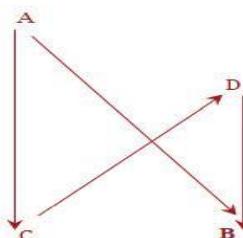
- In a biological system the endergonic processes proceed by coupling to exergonic processes.
- During a chemical reaction, heat is released or absorbed. Enthalpy is a measure of change in heat content of the reactant, when compared to product, which is denoted as ΔH .
- ΔS is the change in entropy. It is the term used to express the degree of randomness or disorder created during a reaction. The randomness is increased when a biomolecule is broken down to smaller molecules. For example:



- In this process there is an increase in randomness because 7 molecules produce 12 molecules. Whenever chemical reaction proceeds, if there is an increase in the number of molecules, then, there is an increase in molecular disorder and thus an increase in entropy. This contributes to $- \Delta G$.
- The relationship between change in free energy, enthalpy and entropy is expressed as

$$\Delta G = \Delta H - T \Delta S$$

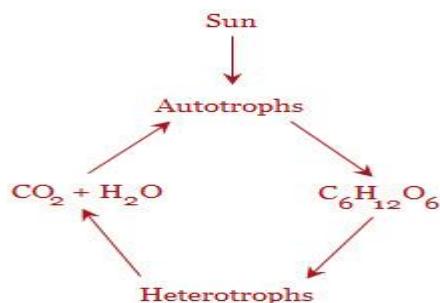
- In this reaction T is the absolute temperature.
- The measurement of absolute heat content and entropy contents of the substrate and the product are difficult. But it is possible to measure the change in these qualities. ΔH can be measured by a calorimeter.
- The conversion of metabolite A to B, occurs with the release of energy, i. e., Exergonic.



- It is coupled to another reaction, in which, free energy is required to convert metabolite C to D (which is an endergonic reaction).
- As some of the energy is liberated in the degradative reaction, it is transferred to the synthetic reaction, in a form other than heat; hence, the term exothermic or endothermic cannot be applied.
- The total of anabolic and catabolic processes is called as “Metabolism”.
- The overall net change in metabolism is exergonic.
- Thus, the ultimate purpose of oxidation is to capture the energy in some intermediate stable form, which could further be used for other biosynthetic processes.
- Not all-biological oxidation-reduction involves oxygen and carbon. Some bacteria use nitrogen for their oxidation-reduction reactions.

AUTOTROPHS AND HETEROTROPHS

- The living organisms have to obtain energy from the diet. The sun is the ultimate source of energy. The organisms can be divided into two major groups viz. autotrophs & heterotrophs:
 - *Autotrophs*: (Self-synthesizing) like plants trap the energy from the sun through chlorophyll and utilize this energy for the synthesis of food particles from CO₂ and H₂O.
 - *Heterotrophs*: (Depending on others) like higher animals, cannot utilize the energy from the sun directly. Instead, they trap energy from the food a particle stored in plants and is used for growth, development and reproduction.



- Life is just electron flow. The flow of electrons in oxidation-reduction reactions is responsible for all work done by living organisms.
 - A chemical reaction, in which electrons are transferred, from one molecule to another is called “oxidation-reduction” or “redox” reactions. Oxidation of one molecule follows reduction of other molecule.
 - The electron-donating molecule is called as a reducing agent (reductant) and the electron-accepting molecule is known as oxidizing agent (oxidant).
 - Oxidation is defined as removal of electrons and reduction is the gain of electrons.



MODE OF ELECTRON TRANSFER

Electrons are transferred from one molecule to another in one of the four different ways as given below:

In the form of hydride ions

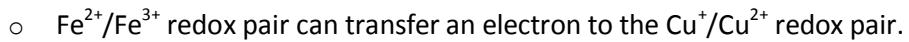
- Hydride ion has 2 electrons, which are transported from electron donor to acceptor.
 - E.g.: Hydride ions (H^-) to the coenzyme NAD⁺

In the form of hydrogen atoms

- A hydrogen atom consists of a proton (H^+) and a single electron (e^-).



- Where AH_2 acts as the hydrogen (or electron) donor. AH_2 and A^- , constitute the conjugate redox pair, which can reduce another compound B by the transfer of hydrogen atoms.
 - E.g.: Hydrogen atoms (H_2) to the coenzymes flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD), or coenzyme Q.
 - **Directly as electrons**



- E.g.: Electrons (e^-) to the cytochromes, which are coupled to oxidative phosphorylation.
 - Direct combination with an organic reductant with oxygen



- Here, hydrocarbon is the electron donor and the oxygen atom is the electron acceptor.

All four types of electron transfer process occur in cells.

The use of oxygen in respiration (aerobic oxidation) is to obtain energy in the form of ATP.

SUB CLASSES OF OXIDOREDUCTASES

Enzymes involved in oxidation and reduction reactions

- The enzymes involved in oxidation and reduction reactions are generally known as oxidoreductases.
 - They are classified into 4 groups. They are
 - Oxidases
 - Dehydrogenases
 - Hydroperoxidases and
 - Oxygenases.

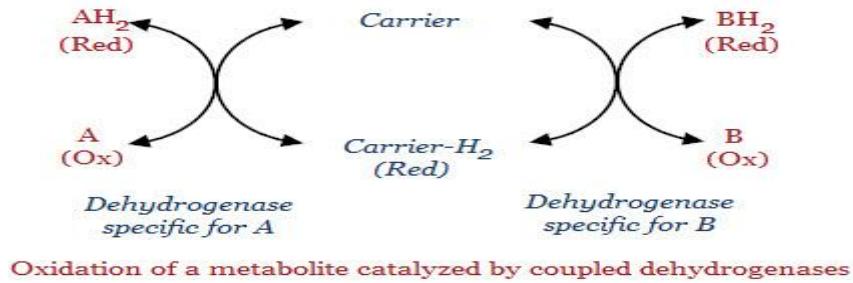
OXIDASES

- Oxidases is the general name for enzymes, that catalyze the removal of hydrogen from a substrate using either molecular or atomic oxygen as a hydrogen acceptor. They form either water or hydrogen peroxide as an end product.
- Eg: Cytochrome oxidase. It is the terminal component of the electron carrier chain found in mitochondria.
- These enzymes are generally present in microsomal and peroxisomal sites.
- Some oxidases use either FMN or FAD as coenzyme for the oxidation of the substrate.
- Eg: L- amino acid oxidases (FMN), Glucose oxidase (FAD) and Xanthine oxidase (FAD)



DEHYDROGENASES

- Enzymes, which remove the pair of hydrogen atoms from the substrate using coenzymes, like NAD⁺, NADP⁺, FAD and FMN as hydrogen acceptors.
- These enzymes cannot use oxygen as hydrogen acceptor.
- These reactions are reversible; so these enzymes allow the hydrogen groups to be freely transferred within the cell.
- In the presence of these enzymes the substrates are oxidized at the expense of another, enabling the oxidative processes to occur in the absence of oxygen. Many biological oxidation reactions are dehydrogenations.
- E.g.:
 - Glyceraldehyde 3-phosphate dehydrogenase (NAD⁺)
 - Glucose-6-phosphate dehydrogenase (NADP⁺)
 - Succinate dehydrogenase (FAD⁺)
- Except cytochrome oxidase, other cytochromes of the respiratory chains are also classified as dehydrogenases. They are involved in the transfer of electrons.



HYDROPEROXIDASES

- This type of enzymes uses hydrogen peroxide or organic peroxide as the substrate. There are two types of hydroperoxidases:
 - Catalase
 - Peroxidase.
- Catalase
 - The hydrogen peroxide is degraded by the catalase, as follows,



- In the above reaction, one molecule of H_2O_2 acts as electron donor and the other as electron acceptor.
- Peroxidase
 - This enzyme acts on hydrogen peroxide in the presence of an electron acceptor with the formation of water.



- The enzyme glutathione peroxidase, containing selenium as prosthetic group catalyzes the degradation of H_2O_2 .



- Peroxisomes found in many tissues are rich in oxidases and catalases.
- Some of the oxidative reactions catalyzed by oxidases produce H_2O_2 , which is highly toxic. This causes damage to DNA, RNA, proteins and unsaturated fatty acids present

in the cell membrane, resulting in the development of cancer and inflammatory diseases. Hence, the hydrogen peroxide must be removed by conversion into a non-toxic compound like water.

OXYGENASES

- Oxygenases incorporate one or two of the atoms of oxygen into the substrate.
- Accordingly, oxygenases may be divided into 2 sub-groups. They are.
 - Monoxygenases.
 - Dioxygenases.
- Monoxygenases
 - Monoxygenases incorporate one atom of O₂ into the substrate and the other oxygen atom will be reduced to H₂O.
 - Hence, they need two substrates to serve as reductants.
 - They are also called as “hydroxylases”, “mixed function oxidases” or “mixed function oxygenases”.



- Microsomal cytochrome P-450 monooxygenase systems are important for the hydroxylation of many drugs.



- Mitochondrial cytochrome P-450 monooxygenase systems catalyze the hydroxylation of many physiological compounds such as steroids and fatty acids.

- Dioxygenases
 - These are enzymes that incorporate both the atoms of oxygen into the substrate.

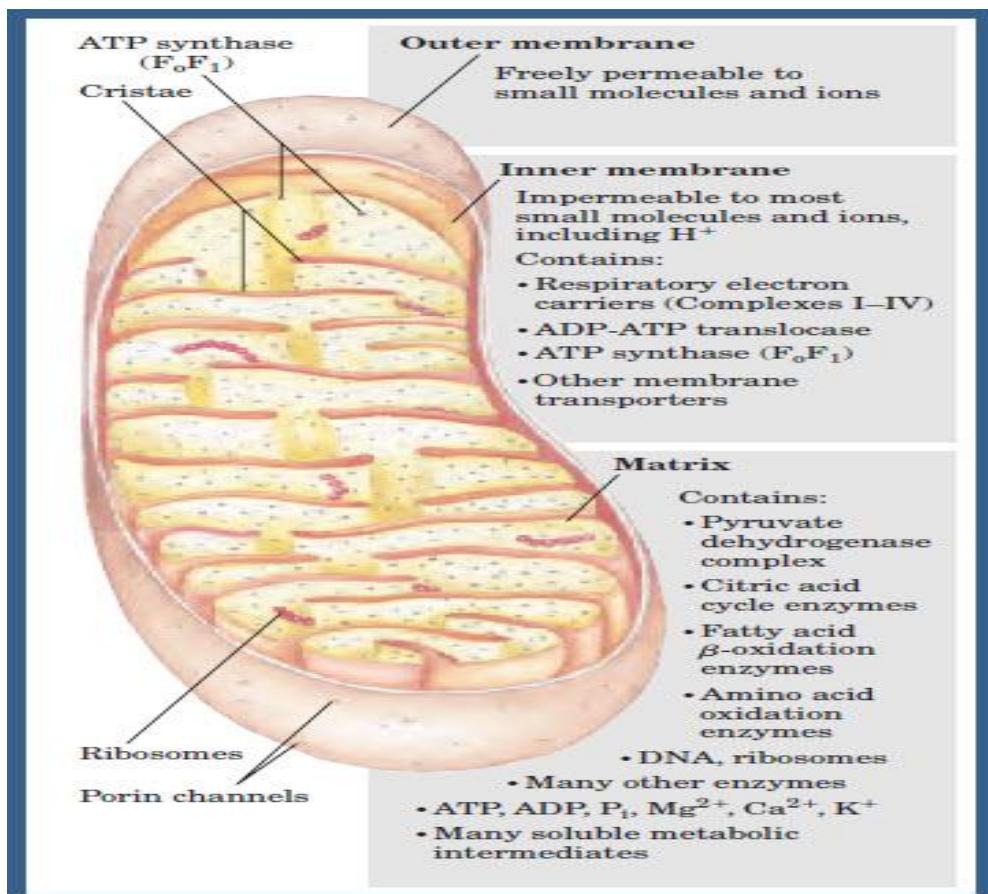


- They are used in the conversion of arachidonate into prostaglandins, thromboxanes and leukotrienes.
- They are also used in the carbon - carbon cleavage in the degradation of amino acids.
- E.g.: Tryptophan dioxygenase catalyzes the opening of the five membered ring of tryptophan.

ELECTRON TRANSPORT CHAIN

MITOCHONDRIA

- It is the powerhouse of the cells. They are about 1 μm in diameter.
- The size, shape and number vary depending upon the location and species.
- Each mitochondrion has two membranes, an outer membrane and an inner membrane.
- The outer membrane is simple, composed of about 50% lipid and 50% protein. It is permeable to most small molecules.
- The inner membrane is structurally and functionally complex and shows a highly selective permeability. It contains roughly about 80% proteins.
- The space between outer and inner membrane is known as "inter-membrane space" to which protons are pumped out from the matrix, during electron transport.
- The inner membrane has infoldings known as "cristae", which increases the surface area of the membrane and it separates the internal matrix from intermembrane space.
- The inner membrane contains the electron carriers for electron transport chain (ETC), and the enzyme, ATP synthetase for oxidative phosphorylation, and also several transport proteins.
- The matrix contains enzymes, involved in citric acid cycle, β -Oxidation of fatty acids, urea cycle and haem synthesis.



MITOCHONDRIAL ELECTRON TRANSPORT CHAIN

- During the enzymatic oxidation of carbohydrates, fatty acids and amino acids, the “Reducing equivalents” such as NADH and FADH₂ containing electrons are produced.
- The electron transport chain oxidizes the above coenzymes by removing electrons and carries the electrons through the carrier system and finally the electrons are donated to O₂, which is reduced to H₂O.
- This whole process is carried out in a series of reactions, which causes gradual release of free energy that could be captured in the chemical form like ATP.
- If NADH or FADH₂ donate the electrons directly to molecular O₂, there would be a large free energy release, which cannot be captured in any stable chemical form.
- Various complexes of the electron transport chain present in the lipid bilayer, which enables the carrier to transport electrons.
- The energy released during the electron transferring process is utilized for the phosphorylation of ADP to ATP by ATP Synthase.
- The process of electron transfer followed by ATP synthesis is known as “oxidative phosphorylation”.

COMPONENTS OF RESPIRATORY CHAIN

- The components of Respiratory Chain are:
 - Cytochromes
 - Flavoproteins
 - Fe –S protein
 - Co-enzyme Q

Cytochromes

- Cytochrome is a class of haemoprotein (an iron-containing haem group attached to protein).
- These “cell pigments” are present in all living tissues that require oxygen.
- The amount varies proportional to the respiratory activity of the tissue.
- They are involved in the transfer of electrons in association with a reversible change in oxidation state of the haem protein (ferrous (Fe²⁺) to Ferric (Fe³⁺)).
- There are 4 major classes of cytochromes, viz., a, b, c and d.
- The members of each subclass are distinguished from each other through numerical subscripts and by their characteristic wavelength of absorption maximum. E.g. Cytochrome C₁, Cytochrome C₅₅₅.
- They also differ from each other through their type of haem group.
- Cytochrome oxidase (aa₃) is the site of cyanide inhibition and also by azide, CO and H₂S.

Flavoproteins

- The enzyme “dehydrogenase”, which removes electrons from NADH, or from substrates like succinate, contains flavins as prosthetic group. Flavins are referred to riboflavin (Vitamin -B₂).
- There are 2 enzyme complexes,

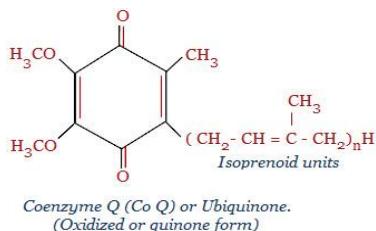
- NADH – dehydrogenase, which contain FMN (Flavin mononucleotide),
 - Succinate dehydrogenase, containing, FAD (Flavin adenine dinucleotide).
- These 2 dehydrogenases are present in the inner mitochondrial membrane. Apart from these two, there are 9 other flavoprotein dehydrogenases, present in mitochondria (8 are in matrix and one in the inner mitochondrial membrane, i.e., Glyceraldehyde- 3- phosphate dehydrogenase).

Fe –S protein

- These proteins contain Fe atoms that are bound to the S-atom of the cysteine residues of the protein.

Co-enzyme Q

- Co- enzyme Q is a quinone derivative with a long isoprenoid side chain.
- It is also called as “Ubiquinone”, because, it is present ubiquitously (ubiquitous = present everywhere) in all biological systems.
- CoQ can accept hydrogen atoms both from FMNH_2 , produced by NADH dehydrogenase and from FADH_2 , which is produced by succinate dehydrogenase.
- It is a lipid soluble compound and therefore freely diffusible within the inner membrane.
- It is capable of undergoing reversible redox reactions.

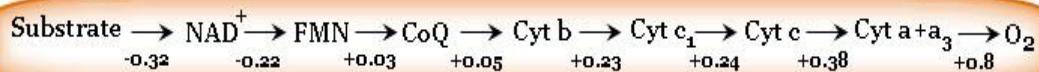


ARRANGEMENT OF COMPONENTS OF ETC

Sites of ATP production

- The arrangement of these carriers is based on the tendency of each molecule to accept or release electrons, i.e., reduction potential (E_{o}^{\prime}).
- Reduction potential is a measure of energy released, when the compound accepts electrons. Its value is expressed in terms of volts. It shows the willingness of the compound to accept electrons.
- More the positive value of reduction potential, more the willingness to accept electrons (oxidant).
- More the negative value, more the tendency to donate electrons (reductant).
- Electrons move spontaneously in forward direction, from the molecule with more negative charge to the molecule with more the positive charge.
- Thus, the molecules with more negative reduction potential are arranged on the extreme left (NADH). As you proceed to the right of the chain, the positive charge increases gradually and it reaches the maximum at the extreme right (O_2).

- The electron flow through this electron transport chain is an irreversible process.
- The components of the respiratory chain are found in the inner mitochondrial membrane.



- The Electron transport chain has been separated into 5 components or complexes, viz.,
 - Complex I. NADH dehydrogenase
 - Complex II. Succinate dehydrogenase
 - Complex III. Ubiquinol dehydrogenase
 - Complex IV. Cytochrome oxidase
 - Complex V. ATP synthase

Complex I: NADH dehydrogenase

- This enzyme contains covalently bound FMN. The $\text{NADH}^+ + \text{H}^+$ from the mitochondrial matrix, causes the reduction of FMN to FMNH_2 . This FMNH_2 , then reduces the UQ to UQH_2 through Fe-S protein.
- The UQH_2 , then diffuses out to complex III. In this course of electron transfer, 4 protons are pumped out from the matrix into the intermembrane space.

Complex II: Succinate dehydrogenase

- This is an enzyme of TCA cycle. It oxidizes succinate into fumarate in which, the covalently bound FAD of the enzyme, is converted into FADH_2 . This FADH_2 reduces UQ into UQH_2 . This UQH_2 also, diffuses out to complex III. Here, no protons are pumped out.

Complex III: Ubiquinol dehydrogenase or Cyt bc₁ complex

- The UQH_2 from complex I and II donate its electrons to cyt.b. Thus, cyt b gets reduced. Then, this reduced cyt b donates its electrons to cyt c₁, then in turn, to cyt c. During this electron transfer, 4 protons are pumped out into the intermembrane space. The cyt c is water soluble, loosely attached molecule.

Complex IV: Cytochrome oxidase

- It contains cyt a and a₃. The electrons from reduced cyt c are passed to cyt a and then to cyt a₃. This cytochrome is the only electron carrier in which, the haem iron has a free ligand that can react directly with molecular oxygen. Here, the reduction of O_2 with electrons and protons results in the formation of H_2O . Reduction of one molecule of O_2 to 2 H_2O requires the addition of four electrons. In this electron transfer also, 2 protons are pumped out.

Complex V: ATP synthase

ATP synthase is a multisubunit enzyme composed of several polypeptides. It is also called $F_0 - F_1$ ATP synthase. It contains the base F_o and the stalk connecting the headpiece, F_1 . They are mushroom shaped unit seen as a projection (head piece is pointing towards the matrix) from the inner mitochondrial membrane. F_1 component contains the catalytic subunit. F_0 component is a proton channel (F_0 indicates its sensitivity to oligomycin, an antibiotic preventing the entry of protons and thereby inhibiting ATP synthesis).

- The passage of protons from the intermembrane space to the matrix through the complex V energizes the enzyme, which synthesizes the ATP from ADP and P_i .

Complex	No. of protons pumped out
Complex I	4
Complex II	--
Complex III	4
Complex IV	2
Complex V	--

- So, the NADH dehydrogenase (FMN) – dependent oxidation pumps out 10 protons.
- The FAD- dependent dehydrogenase pumps out 6 protons. For example succinate dehydrogenase (which uses FAD as coenzyme, enters through complex III).
- Thus, 1 ATP is synthesised for every 4 protons entering the matrix through F_0 - F_1 ATP synthase system.
- The net yield of ATP per one molecule of NADH oxidized is 2.5 and for one molecule of $FADH_2$ oxidized is 1.5.

Complex	Name	No. of protons pumped out	Function
I	NADH dehydrogenase	4	Reduction of UQ by NADH
II	Succinate dehydrogenase	--	Reduction of UQ by succinate
III	Ubiquinol dehydrogenase	4	Transfer of electrons from UQH_2 to Cyt-C
IV	Cytochrome oxidase	2	Transfer of electrons from Cyt-C to O_2

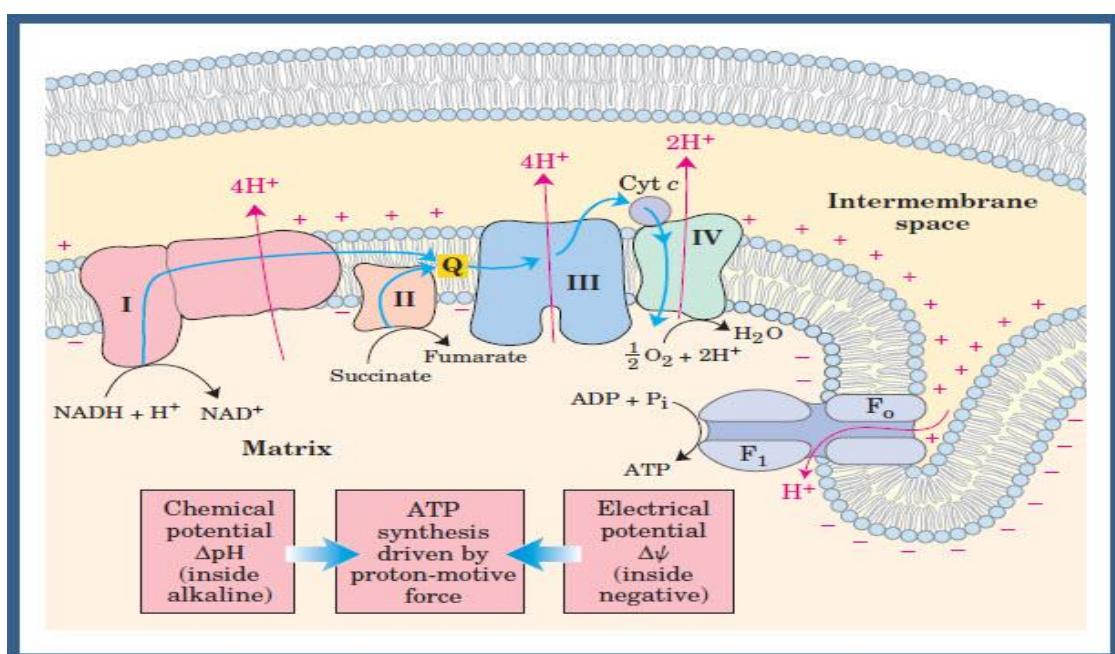
- The process of oxidative phosphorylation is explained through the chemi-osmotic hypothesis proposed by Peter Mitchell.

CHEMI-OSMOTIC HYPOTHESIS

- The process of oxidative phosphorylation could be explained through chemi-osmotic hypothesis.

CHEMI-OSMOTIC HYPOTHESIS OF OXIDATIVE PHOSPHORYLATION

- It is also known as “Mitchell’s hypothesis” (1961).
- He proposed that the electron transport in the respiratory chain results in the movement of protons across the membrane from the mitochondrial matrix into the intermembrane space.
- Due to proton movement the inner matrix becomes alkaline (increase in pH) and becomes more negatively charged.
- This process creates an electrical (proton) gradient (more positive charge in the intermembrane than the matrix).
- When the protons are transported back to matrix through the ATP synthase complex, it gets energized. The activated enzyme catalyses the synthesis of ATP from ADP and inorganic phosphate.
- This explains that chemical reaction is driven by movements of molecules or ions between osmotically different spaces separated by membranes.
- For every 4 protons that are transported back into the matrix via the Fo-F1 ATP synthase, one ATP is synthesized from ADP and Pi.
- Thus, NADH-dependent dehydrogenase provides 2.5 ATP (by 10 protons) and FAD-dependent dehydrogenase provides 1.5 ATP (by 6 protons).



INHIBITORS OF ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

- Some compounds block at any point in the respiratory chain, preventing oxygen consumption and ATP production. There is a reduction in the performance of TCA cycle.
- Site specific (sites where ATP synthesis occur) and Non-site specific inhibitors:

Site specific inhibitors

- Compounds rotenone (a fish poison), barbiturates and piericidin A (an antibiotic), bind with Complex I and prevent electron transfer from Fe-S protein of Complex I to CoQ.
- Antimycin A inhibits transfers of electrons through Complex III (between cytochrome b and c).
- Cyanide (CN), carbonmonoxide (CO) and H₂S inhibit Complex IV (cytochrome oxidase).

Non-site specific inhibitors

- Oligomycin, an antibiotic binds to the ATP synthase system and inhibits the proton flow, thereby preventing the conversion of ADP to ATP.
- The compound atractyloside inhibits ATP-ADP translocase enzyme.

UNCOUPLERS OF ETC

Uncouplers: The compounds that allow the electron transport to continue, but prevents the phosphorylation (ATP synthesis), are known as uncouplers.

E.g. addition of 2,4-dinitrophenol allows diffusion of protons from the intermembrane space to matrix without energizing the ATP synthase complex. Hence, ATP production does not occur, as proton gradient across the inner mitochondrial membrane is not maintained and energy is released as heat.

- Similar mechanism occurs in brown adipose tissue (brown fat), where the protein “thermogenin”, allows protons to bypass the Fo-F1 ATP synthase. The energy released as heat, helps to maintain the body temperature in hibernating (winter sleep) animals and in new born infants.

P/O ratio:

- The number of molecules of ATP formed per pair of electrons transferred down the respiratory chain to atomic oxygen is termed P/O ratio.
- When substrates are oxidized via NAD-linked dehydrogenase in the respiratory chain, 2.5 molecules of inorganic phosphate are incorporated into 2.5 molecules of ADP, to form 2.5 molecules of ATP per atom of oxygen consumed. Hence, the P/O ratio is = 2.5.
- When a substrate like succinate is oxidized via a flavoprotein – linked dehydrogenase, only 1.5 molecules of ATP are formed. i.e., P:O = 1.5

Control of Respiratory Chain

- The conditions that control the rate of respiration in mitochondria are as follows:
 - The capacity of the respiratory chain itself, when all substrates and components are present in required amounts.
 - Availability of ADP.
 - Availability of oxygen.

ENERGY RICH PHOSPHATE COMPOUNDS

- These compounds yield energy on hydrolysis.
- Energy rich phosphate compounds can be divided into two groups, based on the release of free energy on their hydrolysis. They are:
 - Low energy phosphate compounds and
 - High energy phosphate compounds.
- *Low energy phosphate compounds* on hydrolysis yield less than -7.0 Kcal/mol. For e.g., glucose 6-phosphate (-3.3 Kcal/mol) and glycerol 3-phosphate (-2.2 Kcal/mol).
- *High-energy phosphate compounds*: These compounds release more than -7.0 Kcal/mol on hydrolysis. E.g., ATP, GTP, CTP and UTP. The two terminal phosphoryl group of ATP are energy rich or high -energy bonds. ATP links the reactions requiring energy, with those reactions that release energy.
 - ATP (\rightarrow ADP +Pi) -7.3 Kcal/mol
 - ADP (\rightarrow AMP +Pi) -7.8 Kcal/mol
 - PPi (\rightarrow 2Pi) -4.0 K cal/mol
 - Creatine phosphate -10.3 Kcal/mol
 - Phosphoenolpyruvate -14.8 Kcal/mol

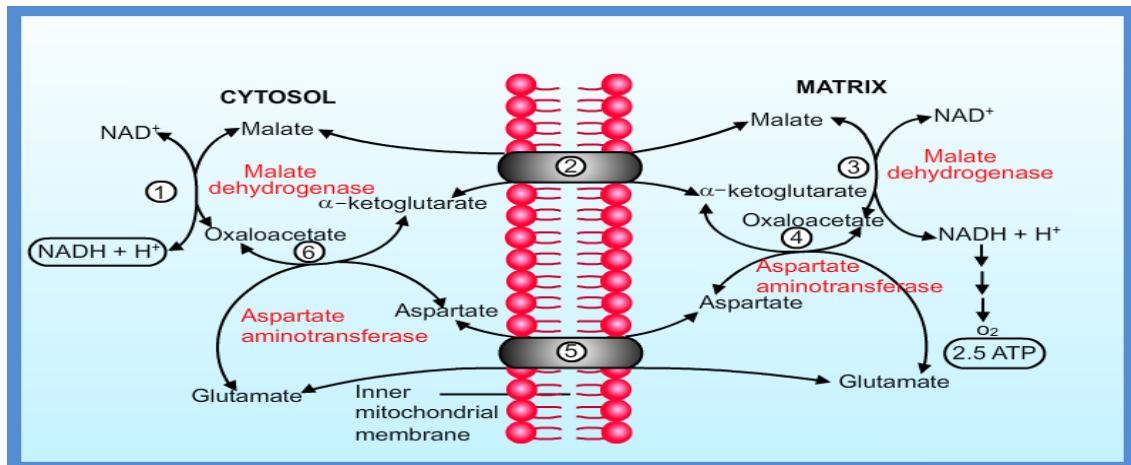
MITOCHONDRIAL SHUTTLE SYSTEMS

- Inner mitochondrial membrane is impermeable to certain important compounds like NAD⁺/NADH/ NADP⁺/NADPH, Coenzyme-A and oxaloacetate.
- NADH is generated in the glycolytic process in cytosol (in the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase).
- It has to be transported across the mitochondrial membrane to get re-oxidized to NAD⁺ via the respiratory chain in mitochondria.
- As the inner membrane is impermeable to NADH, they are transported through special transport system called “shuttle system”.
- Such special systems carry the reducing equivalents (NADH) from the cytosol to mitochondrial matrix by an indirect route.
- There are 2 common and important shuttle systems, viz.,

1) Malate-aspartate shuttle

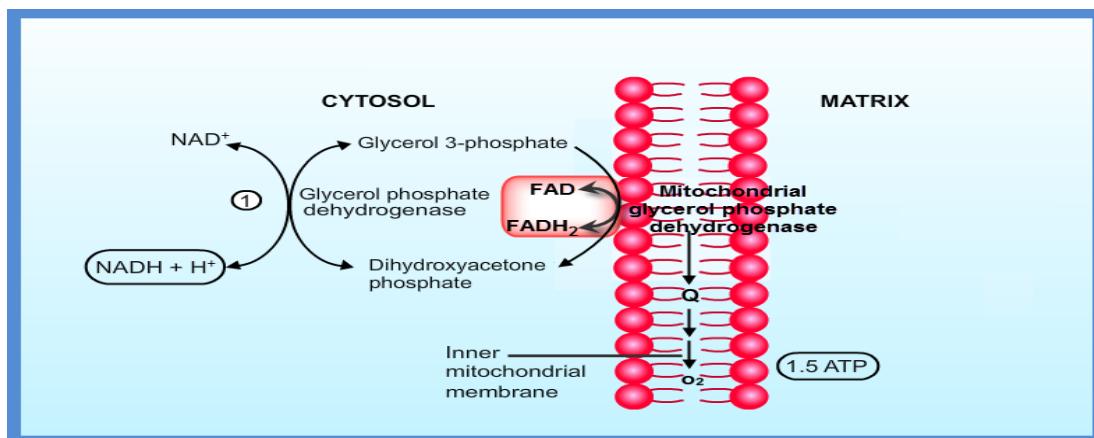
- This functions in liver, kidney and heart mitochondria. It is reversible in nature.
- In this system, the cytosolic NADH is transferred to cytosolic oxaloacetate to yield malate and NAD⁺, by the action of cytosolic malate dehydrogenase.
- The malate thus formed passes through the inner membrane into the matrix via the malate- α -ketoglutarate transport system.

- Within the mitochondrial matrix, the reducing equivalents are passed to matrix NAD⁺, by the action of matrix malate dehydrogenase, thus forming NADH and oxaloacetate in the matrix.
- Then, this NADH passes the electrons to the respiratory chain through complex I (NADH dehydrogenase) producing 2.5 moles of ATP.



2) Glycerol-3-phosphate shuttle

- This system functions in brain, adipose tissues and skeletal muscle. It is irreversible in nature.
- In this system, the cytosolic NADH are transported through glycerol-3-phosphate.



- This glycerol-3-phosphate is oxidized by the enzyme, glycerol-3-phosphate dehydrogenase present in the cytosolic side of the inner mitochondrial membrane. This enzyme has FAD as its coenzyme.
- The reducing equivalents are straight away taken up by FAD to form FADH₂, which then transfers the electrons to Complex III via CoQ.
- Here, the reducing equivalents are not entering the mitochondrial matrix and thus it differs from the Malate - Aspartate shuttle.
- ATP yield is only 1.5 per one mole of cytosolic NADH oxidized.

GLYCOLYSIS

- Carbohydrates form a major portion of food and feed of living organisms and they are the major source of energy for the living cells.
- Digestion of carbohydrates starts in the mouth. In the digestive tracts of animals, carbohydrates are further degraded to yield glucose. Then, the glucose is absorbed into the system and it enters the cell through different glucose transporters, such as Sodium-glucose transporter (SGLUT) in the intestinal luminal cells, and Glucose transporter (GLUT1 to GLUT12) in different tissues.
- Carbohydrates must be oxidized to CO_2 and H_2O , to get the free energy stored in the glucose molecule. Oxidation of glucose starts with glycolysis.
- Carbohydrate is the only source, which provides energy under anaerobic conditions.

Glycolysis

- Glycolysis occurs in cytosol.
- Glycolysis is also known as Embden-mayerhof –Parnas pathway (EMP or EM Pathway) to honour the scientists Gustav Embden, Otto Mayerhof and Jakub Karol Parnas
- It has a sequence of 10 enzyme-catalyzed reactions, where one molecule of glucose (6C) is converted to two molecules of pyruvate (3C).
- Reactions of glycolysis are divided into two phases, preparatory phase and pay off (ATP generating) phase (each containing 5 reactions). Major function of glycolytic pathway is the generation of ATP and intermediates for other metabolic pathways, such as,
 - Synthesis of glycine, serine and cysteine.
 - Synthesis of fatty acid using acetyl CoA, which is a decarboxylated product of pyruvate.
 - Pyruvate can be transaminated to the amino acid alanine.
 - Glycerol 3-phosphate, a component of triacylglycerol is derived from glycolytic pathway.

Anaerobic glycolysis

- It is the oxidation of glucose in the total absence of oxygen.
- Lactate is the end product.
- Here, the NADH formed in the pay off phase is oxidized by converting pyruvate to lactate in the presence of the enzyme, lactate dehydrogenase, having NAD as the coenzyme.
- Net yield is only 2 ATPs.

- It allows the continued production of ATP in tissues that lack mitochondria (for example, red blood cells). Tissues like brain, GI tract, white blood cells, skeletal muscle, kidney medulla, skin and retina of eye also to some extent, depend on anaerobic glycolysis for the ATP production.

Aerobic glycolysis

- Pyruvate is the end product of aerobic glycolysis.
- Since, the process is in the presence of oxygen, the reducing equivalents from 2 NADH molecules generated in the pay off phase, will be transferred to the mitochondrial ETC, via shuttle processes, depending on the tissues.
- The net yield of ATP will be different from that of anaerobic glycolysis.
 - i.e., Glucose to pyruvate - 2 ATPs plus
 - 2 NADH to ETC – 2×2.5 (Malate -Aspartate Shuttle) / 2×1.5 (Glycerol-phosphate shuttle) ATPs as the case may be in different tissues.
- The pyruvate formed in aerobic glycolysis is transported to mitochondria and decarboxylated to acetyl- CoA, which enters the citric acid cycle and is completely oxidized to CO_2 and H_2O .
- The slowing down of glycolysis in the presence of oxygen is called Pasteur effect. In muscle, the rate of conversion of glucose to pyruvate is much higher under anaerobic condition.

Significance

- Erythrocytes use only glucose *via* anaerobic glycolysis even in aerobic condition which always terminates in lactate, for getting energy. Due to the absence of mitochondria, fatty acids and ketone bodies cannot be metabolized in RBC.
- 1,3-bisphosphoglycerate produced in glycolysis is converted to 2,3-bisphosphoglycerate (2,3 BPG is not a high energy compound) by bisphosphoglycerate mutase. This compound plays a key role in the binding of oxygen to hemoglobin. This cycle in erythrocytes is called as “Rapaport-Leubering Cycle” (a supplementary cycle to Glycolysis).
- 2,3 BPG stabilizes reduced haemoglobin, thereby causing the release of oxygen from haemoglobin.
- Part of glucose entering RBC is shunted through 2,3 BPG cycle, where the net ATP yield is zero.
- Inhibition of respiration by glycolysis is known as Crabtree effect. It occurs in renal medulla and leukocytes an effect, which is opposite to Pasteur Effect - i.e. Inhibition of glycolysis by Oxygen (*via* respiration).
- Inherited aldolase-A deficiency and pyruvate kinase deficiency in erythrocytes, causes hemolytic anaemia.

REACTIONS OF GLYCOLYSIS

- **A) Phase I - Preparative phase**
 - Glucose is phosphorylated twice by ATP and cleaved into 2 triose phosphates.
 - Two ATPs are utilized.
- **B) Phase II - Pay off phase / ATP generating phase**
 - Triose phosphates are oxidized to produce 4 ATP molecules.
 - Additionally, under aerobic condition, the reducing equivalents from 2 NADH molecules generated will be transferred to the mitochondrial ETC, via shuttle processes, depending on the tissues, producing $2 \times 2.5 / 2 \times 1.5$ ATPs.

Important Terms used in Glycolysis:

1) Mutase:

Enzymes that catalyze the transfer of a functional group from one position to another on the same molecule

2) Kinase

Enzymes that catalyze the transfer of a terminal phosphate group from ATP to some acceptor
eg: a hexose sugar in case Hexokinase.

3) Substrate level phosphorylation

Synthesis of ATP from ADP & Pi, when one high energy substrate is converted to another substrate without the involvement of Electron transport chain in the mitochondria.

Eg: Conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate

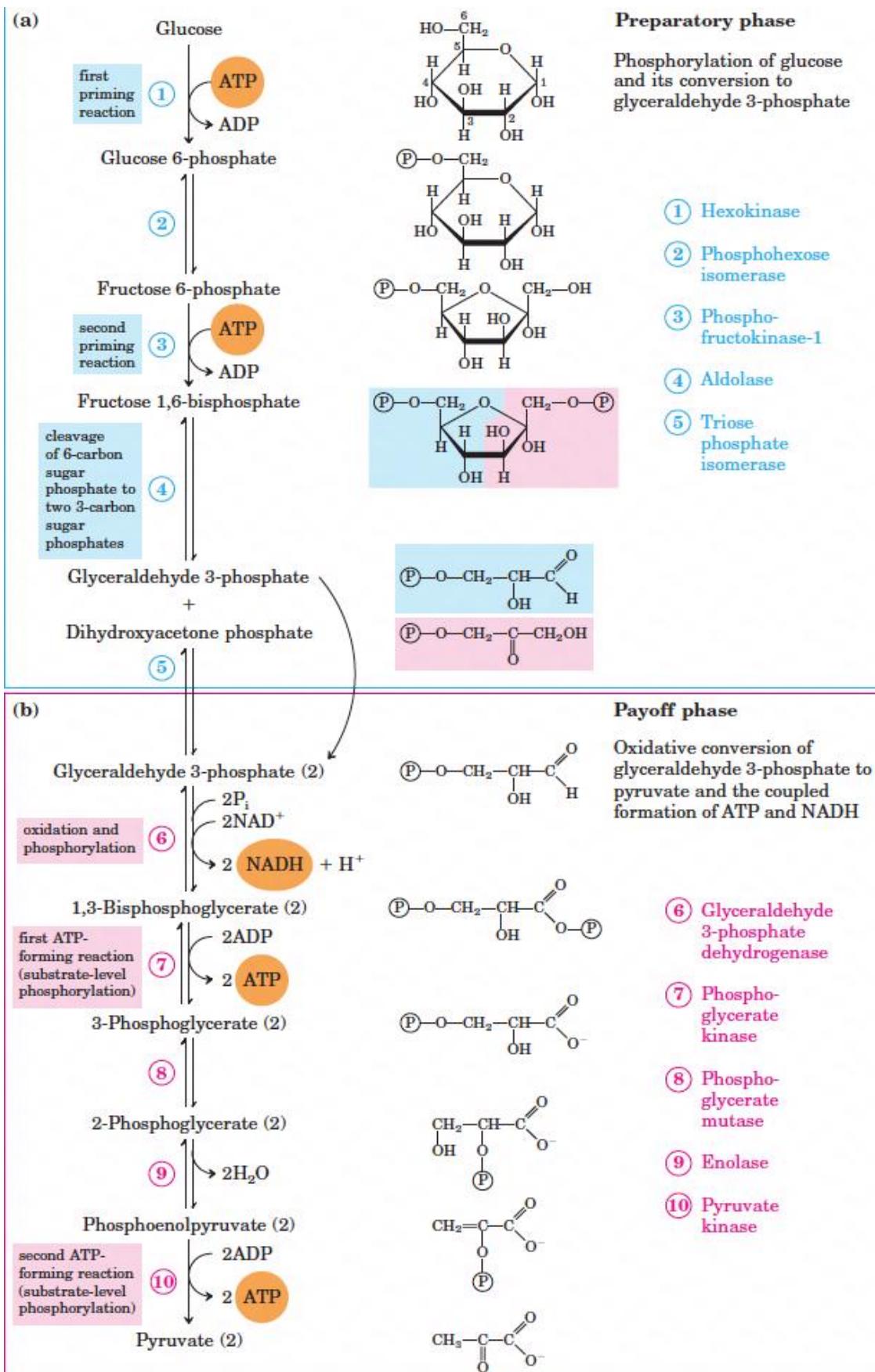


Fig: REACTIONS OF GLYCOLYSIS

Preparatory Phase

- **1st reaction - Phosphorylation**

- Glucose is phosphorylated by ATP on carbon 6 to produce glucose-6-phosphate.
- Catalysed by either hexokinase or glucokinase depending on the concentration of glucose and type of tissue.

Hexokinase	Glucokinase
Present in all tissues	Only in liver
Acts on all hexoses except galactose	Acts only on glucose
Low Km (0.1mM) for glucose	High Km (5mM) for glucose
Inhibited by glucose-6-phosphatase	Not inhibited by glucose-6-phosphatase
Insulin has no action	Insulin stimulates glucokinase

- Reaction is irreversible.

- **2nd Reaction - Isomerisation**

- Glucose-6-phosphate is isomerised to fructose-6-phosphate.
- Catalysed by phosphohexo-isomerase.
- Reaction is reversible.

- **3rd Reaction - Second phosphorylation**

- Fructose-6-phosphate is phosphorylated by ATP to fructose-1,6-bisphosphate.
- Catalysed by phosphofructokinase-1 (PFK-1).
- An irreversible reaction.
- This reaction is the most important control point in glycolysis.

- **4th Reaction - Cleavage of 6C compound into two 3C compounds (triose)**

- Fructose-1,6-bisphosphate is cleaved at the middle to yield one molecule of dihydroxy acetone phosphate and one molecule of glyceraldehyde-3-phosphate.
- Enzyme is aldolase.
- The reaction is reversible.

- **5th Reaction - interconversion of triose phosphates**

- Dihydroxy acetone phosphate is isomerised to glyceraldehyde-3-phosphate for further metabolism in the glycolytic sequence.
- This results in the production of two molecules of glyceraldehyde-3-phosphate from one molecule of fructose-1,6-bisphosphate.

- Enzyme is triose phosphate isomerase.
- Reaction is reversible.

Pay off phase / ATP generating phase

- **6th Reaction - Phosphorylation (from inorganic phosphate, not from ATP)**
 - Glyceraldehyde -3-phosphate is converted to 1,3-bisphosphoglycerate.
 - enzyme is glyceraldehyde -3-phosphate dehydrogenase.
 - This enzyme uses NAD⁺ and inorganic phosphate.
 - From 2 molecules of glyceraldehyde -3-phosphate, 2 NADH are formed.
 - It is also the first reaction in which a high-energy phosphate compound, 1,3-bisphosphoglycerate is formed.
 - Arsenate, which closely resembles the structure of Pi, inhibits the formation of 1,3-bisphosphoglycerate by the enzyme glyceraldehyde -3-phosphate dehydrogenase.
 - Mercury and iodoacetate react with sulphydryl groups of glyceraldehyde -3-phosphate dehydrogenase thereby inhibiting the reaction.
 - Reaction is reversible.
- **7th Reaction - Substrate level phosphorylation reaction (ATP generating reaction)**
 - Transfer of phosphoryl group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.
 - Phosphoglycerate kinase catalyzes the reaction.
 - Reaction is reversible (this is an unique reaction, wherein a Kinase catalyzed step is reversible).
- **8th Reaction - Shifting of phosphate group**
 - Transfer of phosphate group from carbon 3 to carbon 2 of phosphoglycerate i.e. conversion of 3-phosphoglycerate to 2-phosphoglycerate.
 - Enzyme is phosphoglycerate mutase.
 - Reaction is reversible.
- **9th Reaction - Dehydration and redistribution of energy**
 - H₂O is eliminated from 2-phosphoglycerate to produce phosphoenolpyruvate (PEP).
 - Enolase catalyzes the reaction.
 - Energy in the compound is redistributed and converted to high energy compound.
 - Enolase is inhibited by fluoride by binding to magnesium, which is required for the activity.
 - Reaction is reversible.

- **10th Reaction - Second substrate level phosphorylation**

- Phosphoryl group from PEP is transferred to ADP to form ATP and pyruvate.
- Reaction is catalyzed by pyruvate kinase.
- Third and last irreversible reaction in glycolytic cycle.

ENERGETICS OF GLYCOLYSIS

Anaerobic glycolysis



- Total ATP production in the pay off phase = 4
- Total ATP utilization in the preparatory phase = 2
- Net ATP available = 2

Aerobic glycolysis



- ATP production in the pay off phase = 4
- Oxidation of 2 x NADH via mitochondrial ETC = 2 x 2.5 (3 in old concept) in tissues of liver, kidney and heart muscles and 2 x 1.5 (2 in old concept) in brain and skeletal muscles.
- Total ATP production = 4 + 5 = 9 or 4 + 3 = 7, depending on the tissue.
- Total ATP utilization in the preparatory phase = 2
- Net ATP available = 9 - 2 = 7 or 7 - 2 = 5, depending on the tissue.

Reactions yielding ATP and Utilizing ATP in Glycolytic cycle (aerobic condition)

S. No.	Glycolytic cycle	ATP	
		New	Old
ATP Production			
1	1,3-bisphosphoglycerate to 3-phosphoglycerate (2 molecules)	2.0	2.0
2	Phosphoenolpyruvate to pyruvate (2 molecules)	2.0	2.0
3 *	Glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (2 molecules) - 2 NADH formed is oxidized via mitochondrial ETC (in liver, kidney and heart)	5.0	6.0
	Total	9.0	10.0
ATP Utilization			
1	Glucose to Glucose - 6 - phosphate	1.0	1.0
2	Fructose - 6 - phosphate to Fructose - 1,6 - bisphosphate	1.0	1.0
	Total	2.0	2.0
	Net Available ATP	7.0	8.0

* Under anaerobic condition NADH formed during the conversion of Glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate is oxidized in converting pyruvate to lactate and there is no ATP production as shown in step 3. So net yield of ATP will be 2.

THE FATE OF PYRUVATE

- Pyruvate is reduced to lactate under anaerobic condition by the enzyme lactate dehydrogenase.
- Pyruvate is transported to mitochondria and converted to acetyl CoA by pyruvate dehydrogenase complex (PDH Complex), a major fuel of the citric acid cycle and the building block for fatty acid synthesis.
- Pyruvate can be carboxylated to oxaloacetate by pyruvate carboxylase with simultaneous ATP hydrolysis, since it is an endergonic reaction.
- Pyruvate can also be carboxylated to malate in the presence of maleic enzyme and NADH / NADPH as coenzyme.
- Pyruvate can be aminated to the amino acid, alanine.

PYRUVATE DEHYDROGENASE COMPLEX
(The reaction linking glycolysis and citric acid cycle)

Conversion of pyruvate to acetyl CoA in mitochondria:

- Under aerobic condition, pyruvate (2 molecules) formed in glycolysis is transported to mitochondrial matrix with the help of transporter molecules present in the inner mitochondrial membrane.
- In the mitochondrial matrix, pyruvate (2 molecules) is converted to acetyl CoA (2 molecules of acetyl CoA) in the presence of the enzyme Pyruvate dehydrogenase complex PDH Complex).
- The enzyme PDH complex requires 5 coenzymes (TPP, Lipoamide, CoA, FAD & NAD). The coenzyme lipoamide contains the vitamin lipoic acid linked to ϵ – amino group of lysine (amino acid).
- This is an oxidative decarboxylation reaction, generating $2 \times \text{CO}_2$ and $2 \times \text{NADH}$.
- Both NADH generated, enter ETC producing 2×2.5 ATPs (old concept 2×3 ATPs).
- Reaction is irreversible.
- The acetyl CoA formed will enter the TCA cycle.

Energetics

- $2 \times \text{NADH} = 2 \times 2.5 = 5$ ATPs produced.

CITRIC ACID CYCLE (TCA CYCLE)

- The citric acid cycle is also known as “*tricarboxylic acid cycle*” (TCA) or “*Krebs’s cycle*” (to honour the Scientist sir Krebs) or *the final common pathway*. It was postulated by *Sir Hans Adolf Krebs in 1953*. Since, a few of the intermediates (citrate, cis-aconitate and isocitrate) are having 3 carboxylic groups (COOH), the cycle is called Tricarboxylic acid cycle.
- This is the final common pathway and the cycle starts with the entry of acetyl-CoA produced from carbohydrates, fatty acids and amino acids, which is completely oxidized to CO₂ and H₂O.
- Acetyl-CoA is a thioester. It also serves as the substrate for synthesis of fatty acids, cholesterol, ketone bodies and porphyrins.
- This cycle occurs in mitochondrial matrix and it consists of nine reactions.
- For every turn of the cycle, one molecule of acetyl-CoA is completely oxidized to CO₂ and H₂O.
- The catabolism of acetyl-CoA liberates reducing equivalents like NADH and FADH₂, which on oxidation Through ETC, leads to the synthesis of ATP.
- This process is aerobic, requiring oxygen. Either absence or partial deficiency of O₂, causes total or partial inhibition in the activity of the cycle.
- This cycle also provides precursors for the synthesis of fatty acids (acetyl-CoA), amino acids (oxaloacetate & α-ketoglutarate) and glucose.
- Since, this cycle is involved in both the oxidation / degradation of nutrients and also in the synthetic processes, this cycle is also known as “*amphibolic pathway*” (amphi = both)
- Each turn of the cycle generates 3NADH, 1 FADH₂ and 1 GTP. On entering the ETC, the electron carriers (NADH and FADH₂) yield nine molecules of ATP, and an additional high-energy phosphate bond in the form of GTP. Thus for one turn of the cycle, for every acetyl CoA molecule oxidised, totally 10 ATPs are produced.

REACTIONS OF TCA

- **1st Reaction - Condensation reaction**
 - Acetyl CoA reacts with oxaloacetate to form a 6-Carbon molecule, citrate.
 - Reaction is catalyzed by citrate synthase.
- **2nd Reaction - Dehydration reaction**
 - Citrate is converted to cis-aconitate, by elimination of one molecule of water.
 - Reaction is catalyzed by the enzyme aconitase.
- **3rd Reaction - Hydration reaction**
 - Water combines with cis-aconitate to produce isocitrate.
 - Same enzyme, aconitase catalyzes this reaction too.

- **4th Reaction - Oxidative decarboxylation reaction**
 - Isocitrate undergoes dehydrogenation by the enzyme Isocitrate dehydrogenase to form oxalosuccinate, which is highly unstable and immediately gets decarboxylated to α - ketoglutarate.
 - One molecule of CO_2 is liberated.
 - The enzyme requires NAD^+ , which is reduced to NADH and enters ETC.
- **5th Reaction - Oxidative decarboxylation reaction**
 - α - Ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA (a high energy thioester) and CO_2 is released.
 - The enzyme is α - ketoglutarate dehydrogenase complex (which requires all the 5 coenzymes like PDH complex, viz., TPP, lipoamide, CoA, FAD and NAD) and finally, NAD^+ is reduced to NADH , that enters ETC.
- **6th Reaction - Substrate level phosphorylation reaction**
 - Succinyl-CoA is converted to succinate. Energy released b the breakage of the high energy thioester bond is conserved by the phosphorylation of GDP to GTP.
 - Enzyme is succinate thiokinase.
- **7th Reaction - Oxidation reaction**
 - Succinate is oxidized to fumarate.
 - The enzyme is succinate dehydrogenase. It requires FAD as the coenzyme, which is reduced to FADH_2 and enters ETC.
- **8th Reaction - Hydration reaction**
 - Fumarate is convereted to malate by the addition of water.
 - The enzyme is fumarase.
- **9th reaction - Oxidation reaction**
 - Malate is oxidized to oxaloacetate, where NAD^+ is reduced to NADH and enters ETC.
 - Enzyme is Malate dehydrogenase.
 - The oxaloacetate is now available to accept another molecule of acetyl-CoA.

Compounds that inhibit the enzymes of citric acid cycle

Name of the compound	Name of the enzyme
Fluoroacetate	Aconitase
Arsenite	α -ketoglutarate dehydrogenase complex
Malonate	Succinate dehydrogenase

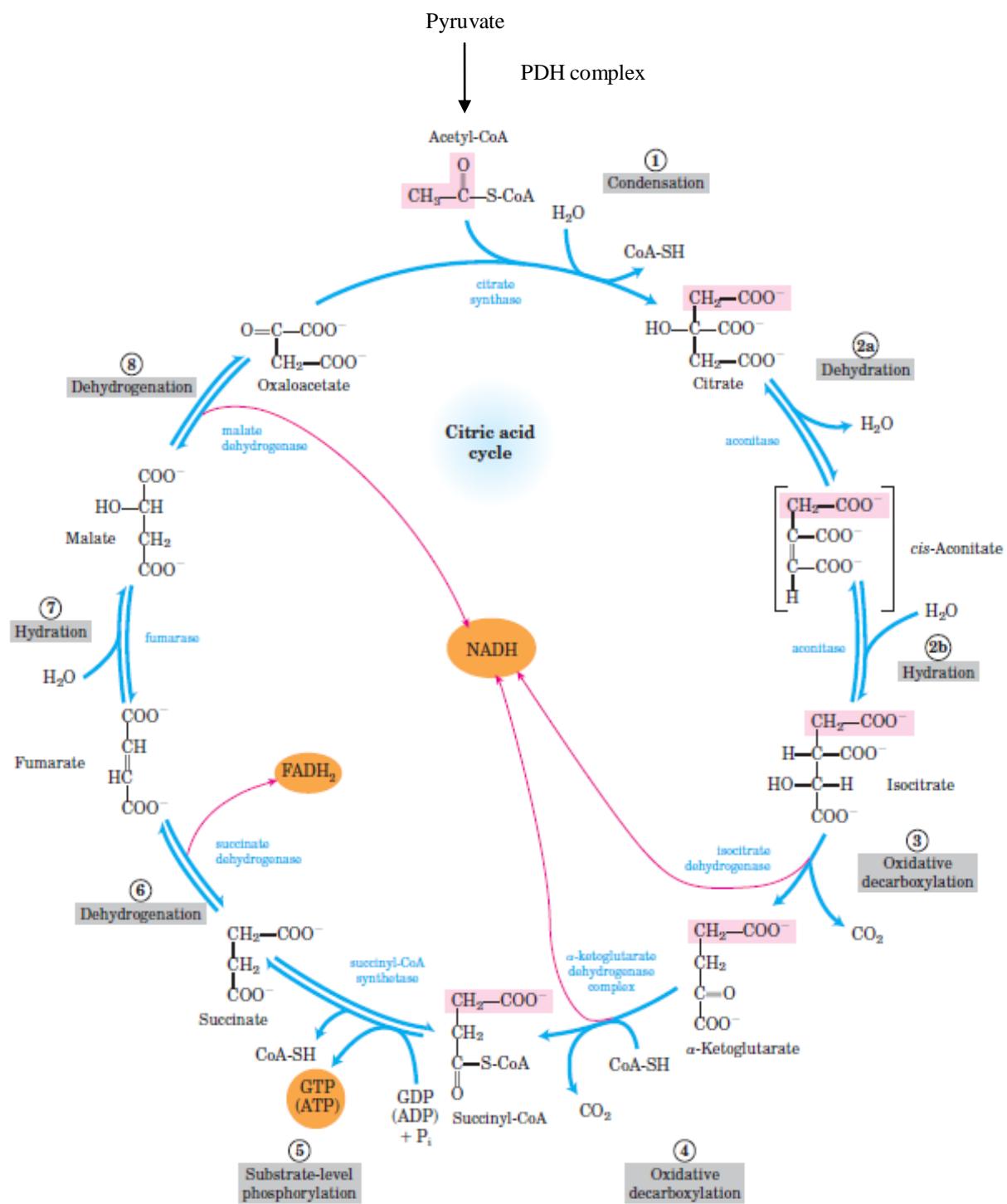


Fig: Reactions of Citric Acid Cycle / TCA cycle

ENERGETICS OF TCA AND COMPLETE OXIDATION OF GLUCOSE

ATP formation in citric acid cycle

S. No.	Reactions	Old concept	New Concept
1.	Isocitrate to α - ketoglutarate (NADH)	3.0 ATP	2.5 ATP
2.	α - ketoglutarate to succinyl CoA(NADH)	3.0 ATP	2.5 ATP
3.	Succinyl CoA to succinate (GTP)	1.0 ATP	1.0 ATP
4.	Succinate to Fumarate(FADH)	2.0 ATP	1.5 ATP
5.	Malate to Oxaloacetate (NADH)	3.0 ATP	2.5 ATP
	TOTAL	12.0 ATP	10 ATP

There are two acetyl CoA. Therefore, $2 \times 10 = 20$ ATP.

Energetics for complete oxidation of one molecule of glucose to CO_2 and H_2O

Process	New Concept	Old Concept
ATP generated		
Glycolysis	4	4
Oxidation of cytosolic NADH through mitochondrial shuttle process (liver, kidney and heart)	5	6
Pyruvate to Acetyl CoA	5	6
Citric acid cycle	20	24
Total	34	40
ATP utilized		
	2	2
Net ATP available		
	32	38

GLUCONEOGENESIS

- Gluconeogenesis is the synthesis of glucose from non-carbohydrate substances.
- The following non-carbohydrate substances can be used for the production of glucose: pyruvate, lactate, propionate, glycerol, and alanine, any metabolite that can be converted to pyruvate or oxaloacetate and intermediates of citric acid cycle.
- This process mainly occurs in liver (90%) and to a smaller extent in renal cortex (10%).
- It is necessary to maintain the normal blood glucose levels for the tissues like central nervous system and red blood cells. These tissues are dependent entirely on glucose for energy. If carbohydrate is not available in sufficient amount from the diet to meet the glucose demand, then glucose will be produced via gluconeogenic process.
- Gluconeogenesis is almost a reversal of glycolysis, wherein the seven reversible reactions of glycolysis are utilized as such, while three of the reactions, i.e. those catalyzed by hexokinase, phosphofructokinase and pyruvate kinase are irreversible and must be bypassed by other alternate reactions.
- The process is endergonic and the synthesis of one molecule of glucose from two molecules of pyruvate consumes 6 molecules of ATP.

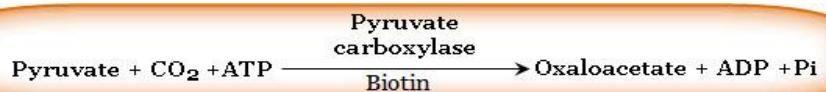
GLUCONEOGENESIS FROM PYRUVATE

Reactions of gluconeogenesis

- The process is occurring in two compartments, the mitochondria and cytosol.
- Considering the reversal of glycolysis from pyruvate to glucose, the first irreversible reaction, which has to be reversed, is the conversion of phosphoenol pyruvate (PEP) to pyruvate.

I. Formation of PEP from pyruvate

- Reactions in mitochondria
- 1st reaction - Carboxylation of pyruvate
 - Pyruvate formed in cytosol is transported to mitochondria, where pyruvate carboxylase converts pyruvate to oxaloacetate.
 - The enzyme requires the presence of acetyl CoA as an allosteric activator and Biotin as Coenzyme.



- 2nd Reaction - Reduction of oxaloacetate
 - Oxaloacetate is converted to malate by malate dehydrogenase (MDH) in the presence of coenzyme NADH. This reaction is occurring in mitochondria, since inner mitochondrial membrane is impermeable to oxaloacetate.



- **Reactions in cytosol**

- Now the malate formed in mitochondria, is transported back to cytosol through the transporter molecule present in the inner mitochondrial membrane.

- **3rd Reaction - Oxidation of malate**

- Malate is converted back to oxaloacetate by malate dehydrogenase - MDH (an isoenzyme present in cytosol).



- **4th Reaction - Decarboxylation of oxaloacetate**

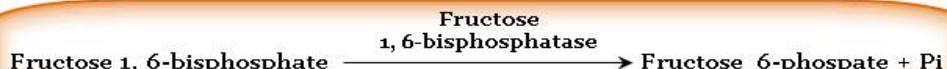
- The oxaloacetate is decarboxylated and phosphorylated to phosphoenolpyruvate by the enzyme phosphoenol pyruvate carboxy kinase (PEPCK).
- In pigeon, chicken and rabbit liver PEPCK is a mitochondrial enzyme and PEP is transported into cytosol for conversion to glucose.
- The reaction is driven by the hydrolysis of GTP.
- Thus the reaction catalyzed by the pyruvate kinase in glycolysis is reversed by the above reactions of gluconeogenesis.



- Rest of the reactions, upto the formation of fructose-6-phosphate are reversible and the next irereversible reaction to be reversed is the conversion of fructose-1,6-bisphosphate to fructose -6-phosphate.
- One important thing to remember in these reversible reactions is that, for the reversible conversion of 3-phosphoglycerate to 1,3-bisphosphoglycerate, phosphorylation is carried out by ATP.

II. Formation of fructose-6-phosphate from fructose-1,6-bisphosphate

- Fructose 1,6-bisphosphate is converted to fructose 6-phosphate, by the enzyme fructose-1,6-bisphosphatase. This reaction by passes phosphofructokinase reaction of glycolysis (the second irreversible reaction).



- Subsequent reactions upto the formation of glucose 6- phosphate are reversible and the last irreversible reaction to be reversed is the conversion of glucose 6-phosphate to glucose.

III. Formation of glucose from glucose-6-phosphate

- Glucose-6-phosphate is dephosphorylated to yield glucose by the enzyme glucose 6-phosphatase. This reaction by-passes the hexokinase reaction of glycolysis (the third irreversible reaction).



ENERGETICS

Energy used in this pathway

- Pyruvate carboxylase - 1 ATP
- Phosphoenolpyruvate carboxykinase - 1 GTP (equivalent to 1 ATP)
- Phosphoglycerate kinase - 1 ATP
- Two pyruvate molecules are required to synthesize one molecule of glucose. Hence, 6 ATPs are needed to form glucose.
- In addition, two moles of NADH are also required per mole of glucose synthesised.
- Net ATP production in glycolysis is only 2 ATPs, while gluconeogenesis consumes 6 ATPs to synthesize a molecule of glucose. Thus, gluconeogenesis is an expensive process in terms of utilization of ATP as energy currency.

GLUCOSE- ALANINE CYCLE

- Pyruvate formed from glycolysis in peripheral tissues can accept amino group from glutamate forming alanine by the process of transamination.
- Also alanine can be formed from protein catabolism in peripheral tissues, especially in skeletal muscles.
- The alanine is transported to the liver, where it undergoes transamination with α -ketoglutarate to reform pyruvate for gluconeogenesis. This process is called glucose- alanine cycle.

GLUCONEOGENESIS FROM GLYCEROL

- The catabolism of triacylglycerols in adipose tissues produces glycerol and acyl-CoA.
- Glycerol is transported to liver, where it is phosphorylated to glycerol 3-phosphate catalyzed by glycerol kinase (this enzyme is absent in adipose tissue).
- Glycerol 3- phosphate enters gluconeogenesis after conversion to dihydroxy acetone phosphate (DHAP), by the enzyme glycerol 3-phosphate dehydrogenase.

GLUCONEOGENESIS - CLINICAL SIGNIFICANCE

- Newborn babies and babies with low birth weight are vulnerable to hypoglycemia. They have reduced amount of adipose tissue to supply free fatty acids or ketone bodies for the production of energy. The gluconeogenic enzymes are also insufficient.
- Similar condition in pigs is common due to low amount of enzymes concerned with gluconeogenesis; this leads to 'Piglet hypoglycemia'
- Blockage of gluconeogenesis due to the deficiency of fructose -1,6 bisphosphatase enzyme prevents the conversion of lactate to glucose causing lactic acidosis.

GLYOXYLATE CYCLE

- Glyoxylate pathway was elucidated by Hans Kornberg and Neil Madsen.
- This pathway is able to convert lipids / acetyl CoA to glucose. It is operating in plants, bacteria and yeast, where acetyl-CoA can be converted to glucose.
- In plant seeds, energy stored as triacylglycerol is converted to glucose at the time of germination. The enzymes of the cycle are present in glyoxysomes. Additionally, glyoxysomes contain all the enzymes needed for fatty acid degradation.
- In animals acetyl-CoA cannot be converted to glucose because a few enzymes unique to glyoxylate cycle, which converts lipids to glucose, are absent.

REACTIONS OF THE CYCLE

- Each turn of the cycle consumes 2 molecules of acetyl CoA.
- Glyoxysomes contain isozymes of TCA cycle enzymes, viz., and citrate synthase and aconitase and malate dehydrogenase.
- Reactions are occurring in three compartments - glyoxysomes, mitochondria and cytosol; the following 4 pathways participate in the conversion of lipid to carbohydrates.

Reactions in Glyoxysomes (Glyoxylate cycle)

- Acetyl CoA (from lipids by β -oxidation of fatty acids) condenses with oxaloacetate to form citrate. The reaction is catalyzed by citrate synthase similar to TCA cycle. Citrate is converted to isocitrate by aconitase.
- Unique to plant cell, where Isocitrate lyase catalyzes the cleavage of isocitrate to succinate and glyoxylate.
- Also unique to plant cells, in which glyoxylate condenses with another molecule of acetyl CoA to yield malate, catalyzed by malate synthase.
- Malate is then oxidised to oxaloacetate by malate dehydrogenase, which is ready for the second turn of the cycle (similar to TCA cycle) and forms Oxaloacetate which can be converted into glucose by gluconeogenic process.
- Similarly Succinate formed in the glyoxylate cycle is transported from glyoxysomes into mitochondria, where it enters the TCA cycle and gets converted to Oxaloacetate which can be converted into glucose by gluconeogenic process.

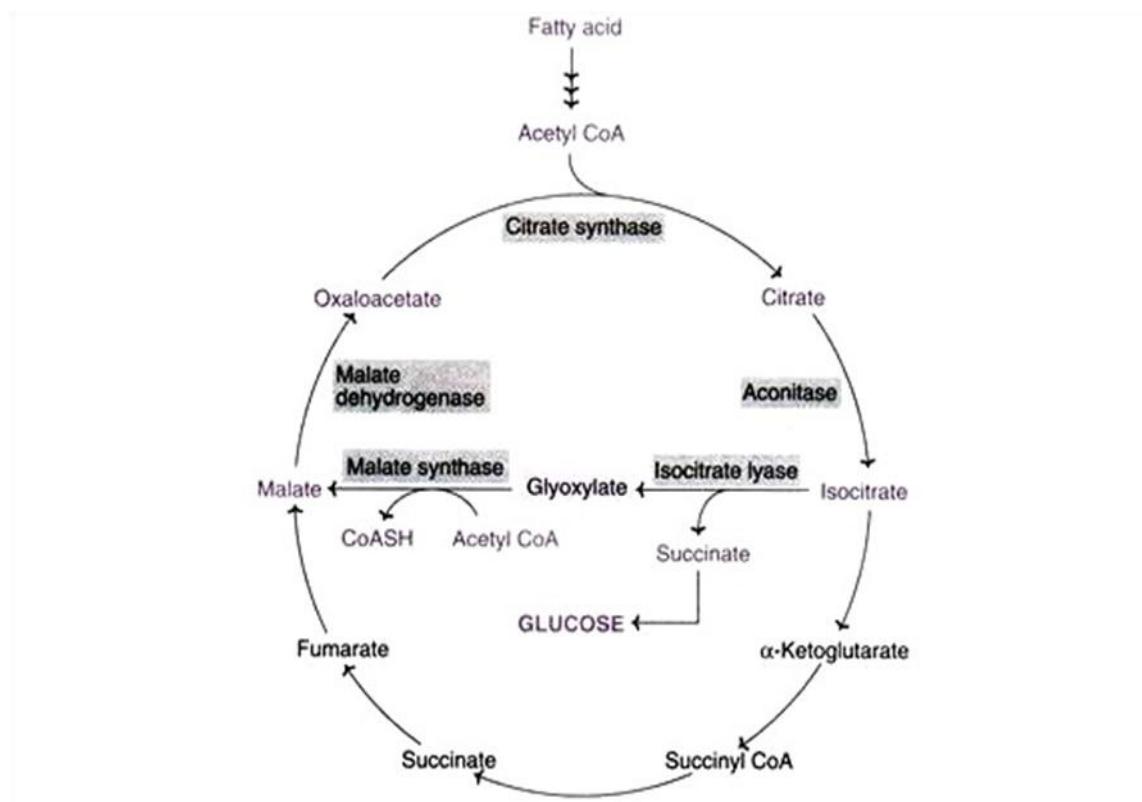


Fig: Glyoxylate cycle

ANAPLEROTIC REACTIONS

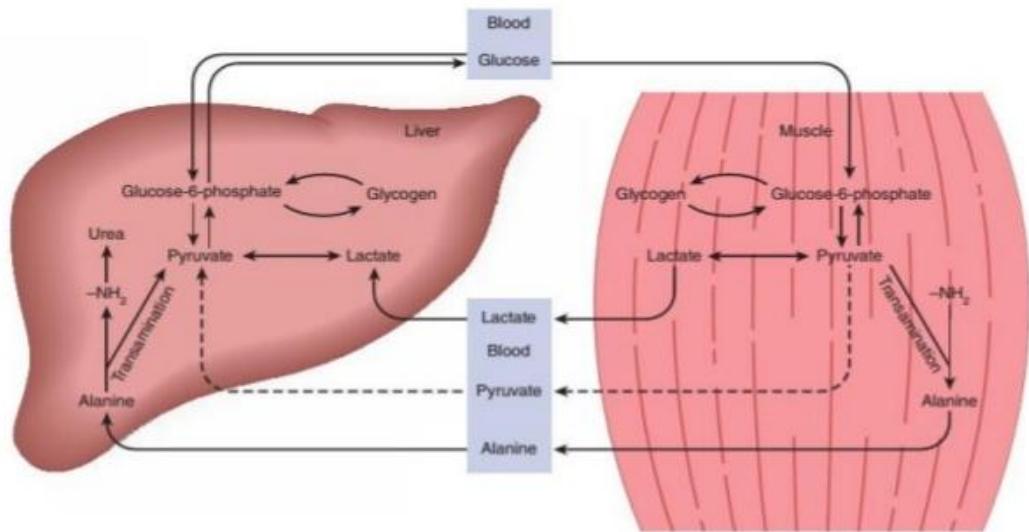
- Some of the biosynthetic reactions use intermediates of citric acid cycle, which results in the decrease in the level of these intermediates in the cycle.
- The decreased level of the intermediates reduces the use of acetyl-Co A for oxidation. So ATP synthesis is hampered.
- Hence, the intermediates of citric acid cycle are replenished by a different set of reactions known as “Anaplerotic reactions”. (Anaplerotic = filling up).
- Eg: Oxaloacetate, one of the intermediates of the citric acid cycle is removed for the synthesis of aspartic acid, to be used in the synthesis of proteins. This causes reduction in the use of acetyl-Co A for oxidation.
- Hence, in another set of reaction, oxaloacetate is produced from pyruvate, by the enzyme pyruvate carboxylase.

AMPHIBOLIC PATHWAY

- In aerobic organisms, the citric acid cycle is an amphibolic pathway (“amphi” means “both”).
- The citric acid cycle serves in both the catabolic and anabolic processes. Such cycles, which are used, for both the purposes of metabolism are termed as “amphibolic pathways”.
- TCA cycle not only functions in the oxidative catabolism of carbohydrates, fatty acids and amino acids, but also provides precursors for many biosynthetic pathways.
- Certain intermediates of the citric acid cycle, particularly α -ketoglutarate and oxaloacetate, can be removed from the cycle to serve as precursors of the amino acids like glutamate and aspartate respectively by simple transamination.
- These amino acids are also used for the synthesis of purines and pyrimidines.
- Likewise, citric acid is used in the synthesis of fatty acids.
- Oxaloacetate is used for the synthesis of glucose.
- Succinyl CoA is used in the synthesis of Haem.

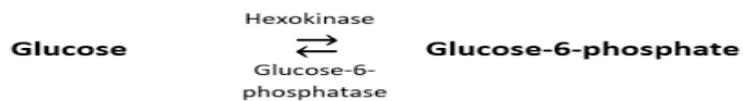
CORI CYCLE AND GLUCOSE- ALANINE CYCLE

- In skeletal muscle and erythrocytes, anaerobic glycolysis result in the production of lactate.
- This lactate enters blood stream and is taken to liver where it is oxidised to pyruvate and is converted to glucose via gluconeogenic pathway.
- The glucose thus formed, enters the blood and is taken up by the skeletal muscle. This reaction is referred to as “Cori cycle” or “Lactic acid cycle”.



FUTILE CYCLE

- This is also known as “ATP-wasting cycle”
- These reactions result in the hydrolysis of ATP without any net metabolic work done.
- Thus, ATP is degraded without any useful work. The energy is liberated as heat.
- This futile cycle are used in small insects like bumble bees, to warm up their flying system to 30°C in cold weather. Under normal circumstances (in a healthy cell), futile cycle does not take place, as the 2 reactions are reciprocally regulated. May occur during metabolic disorders.
- For example, the following reactions of glycolysis and gluconeogenesis.



CARBOHYDRATE METABOLISM IN RUMINANTS

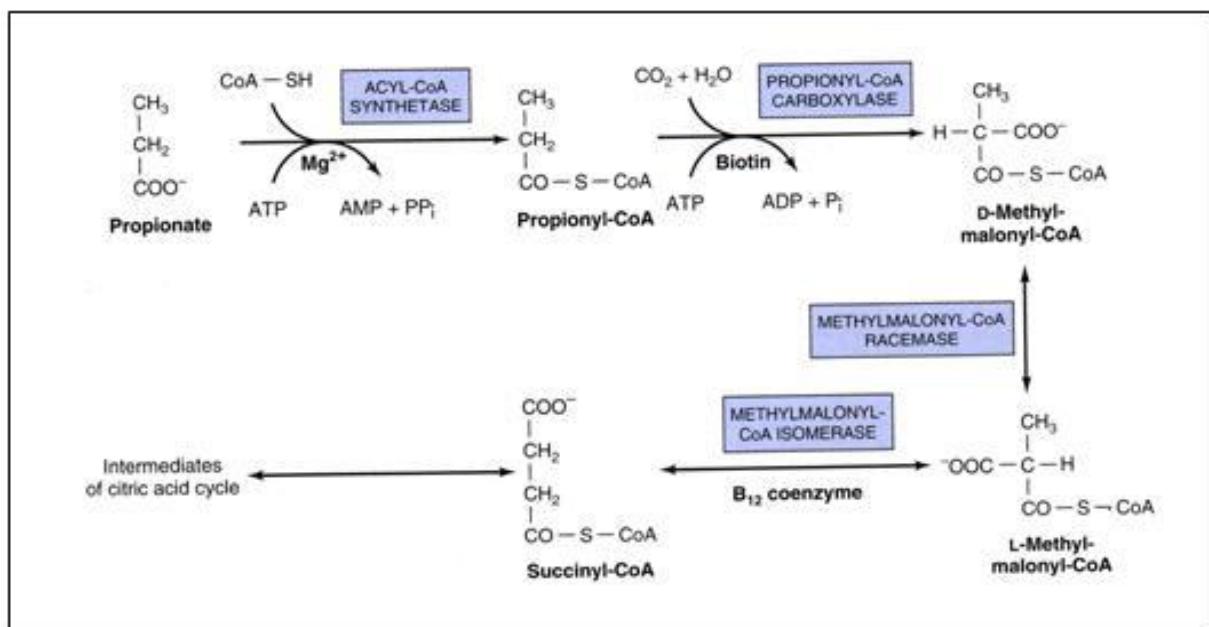
- In ruminants, carbohydrate digestion yields Volatile fatty acids rather than glucose and that is the reason for the normal lower blood glucose level in ruminants when compared to non-ruminants. However, ruminants, like other animals, need glucose not only to supply the brain and other tissues, but also as precursor for *lactose* (*milk sugar*), if they are lactating.
- Ruminant feed comprises of both roughages and concentrates. Roughages, are structural polysaccharides; Cellulose (having β 1,4 - linkage) or hemicellulose (made of xylose), which are degraded by cellulolytic bacteria to *acetate, propionate and butyrate* (VFA). They are in the ratio of 75:15:10, respectively.
- In the case of concentrates based on grains, the main carbohydrate is starch made of α -1,4 linked glucose units, which are rapidly degraded by amylolytic bacteria to acetic (70%), propionic (25%) and butyric acids (5%) and also lactic acid. On feeding starch there is more production of propionate.
- Lactic acid is generally present at low concentrations, which is converted to propionate, by bacteria. It is also metabolized to pyruvate, which can further be converted to glucose or glycogen.
- At low ruminal p^H , the bacteria that convert lactate to propionate are inactivated, resulting in the accumulation of lactic acid, which are absorbed into systemic circulation causing metabolic acidosis. Among the VFA, acetate and butyrate are ketogenic and mainly used for energy production.
- Acetate is also used for the synthesis of fats. Butyrate is converted to β -hydroxybutyrate (ketone body), which is metabolized in the peripheral tissues, for energy production.
- Propionate is mainly used for the production of glucose via gluconeogenesis. In addition to this glucose is also synthesised from glycerol, lactate and protein. In ruminants about 85% of the glucose is formed in liver by gluconeogenesis from non-carbohydrate sources.
- Propionate is the only fatty acid that can be used for glucose synthesis accounting for about 70%. Protein is next accounting for about 20%.

GLUCONEOGENESIS IN RUMINANTS

Conversion of propionate to glucose

- In ruminants, which include cattle, sheep, deer and camel - Propionate is the major source of glucose.
- Propionate is available from rumen fermentation of carbohydrates and also by the β -oxidation of fatty acids with odd number of carbon atoms.

- First is the activation of propionate -
 - Propionate is activated to propionyl-CoA in the presence of ATP and CoA by the enzyme acyl-CoA synthetase.
 - ATP is hydrolysed to AMP and PP_i, with the liberation of energy.
 - The reaction is endergonic.
- Carboxylation of Propionyl CoA -
 - Propionyl-CoA is carboxylated to D-methylmalonyl-CoA in the presence of ATP, by the enzyme Propionyl CoA carboxylase. Enzyme requires Biotin.
 - ATP is hydrolysed to ADP and Pi.
- Conversion of D to L - methylmalonyl CoA.
 - D-methylmalonyl-CoA is converted to L-methylmalonyl-CoA by methylmalonyl-CoA racemase.
- Conversion of L-methylmalonyl CoA to Succinyl CoA -
 - Finally L-methylmalonyl CoA is isomerised to succinyl-CoA, by Methyl malonyl CoA isomerase, which is used for gluconeogenesis.
 - This enzyme requires Vit B₁₂ as a coenzyme.



Clinical significance

- In ruminants supplementation of Cobalt in the mineral mixture is essential for the synthesis of Vit. B₁₂, which is required as coenzyme for methyl malonyl CoA isomerase.
- Vit.B₁₂ deficiency may lead to hypoglycemia in ruminants and to methylmalonyl aciduria in both ruminants as well as in non-ruminants.

HEXOSE MONOPHOSPHATE SHUNT

- Hexose mono phosphate (HMP) shunt is also called as “Pentose phosphate pathway” or “Warburg-Dickens pathway” or “Phosphogluconate pathway”.
- This is an alternate pathway for the glucose catabolism.
- This pathway has 2 major functions:
 - To generate the five carbon sugar, ribose, which is an essential component of nucleotides required for nucleic acid synthesis.
 - To generate NADPH. This is used as reducing powers for the synthesis of fatty acids, cholesterol and other steroids.
- Pathway is active in tissues synthesising fatty acids and steroids such as adipocytes, mammary gland, adrenal cortex, liver etc.
- Pathway is also operational in erythrocytes, because, in these oxygen carrying cells, NADPH plays a protective role against oxidative injury.
- The enzymes of this pathway are located in the cytoplasm.
- The reactions in this pathway, are divided into 2 phases, as
 - Oxidative Phase
 - Non-oxidative Phase.

Oxidative phase

This phase generates NADPH.

- **1st Reaction - Oxidation**
 - Glucose 6-phosphate is converted to 6-phosphogluconolactone by the enzyme glucose 6-phosphate dehydrogenase.
 - Coenzyme NADP is reduced to NADPH.
 - Reaction is irreversible and also regulatory for the entire pentose phosphate pathway.
- **2nd Reaction - Hydrolysis**
 - 6-phosphogluconolactone is converted 6-phosphogluconate, by the enzyme gluconolactone hydrolase (Lactonase).
- **3rd Reaction - Oxidative decarboxylation**
 - Conversion of 6-phosphogluconate to ribulose 5-phosphate.
 - A molecule of CO₂ is released.
 - Enzyme is 6-phosphogluconate dehydrogenase.
 - Here, another (2nd) molecule of coenzyme NADP is reduced to NADPH.

Non-oxidative phase

- **4th Reaction**

- Ribulose 5-phosphate has two fates -
- It can be converted to xylulose 5-phosphate by ribulose phosphate - 3- epimerase or
- To ribose 5-phosphate by ribose phosphate isomerase.
- The remaining reactions of the pathway convert the five-carbon sugar into glycolytic intermediates, which can either regenerate glucose-6-phosphate for further oxidation via glycolytic pathway. The enzymes required are transketolase and transaldolase.

- **5th Reaction**

- xylulose 5-phosphate and ribose 5-phosphate react to form sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate.
- Enzyme is transketolase, which is a thiamin pyrophosphate dependant enzyme that catalyzes the transfer of two carbon (glycol aldehyde) keto group from a keto phosphate to an aldose phosphate.
- i.e.. Two carbon unit from xylulose 5-phosphate is transferred to ribose 5-phosphate, producing the seven carbon ketose, sedoheptulose 7-phosphate.

- **6th Reaction**

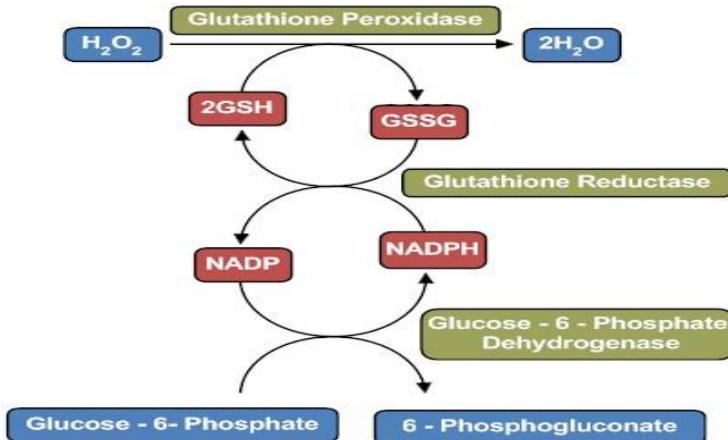
- Sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate react to form fructose 6-phosphate and erythrose 4-phosphate.
- Enzyme is transaldolase, which catalyzes the transfer of a three carbon fragment (dihydroxyacetone moiety) from a keto phosphate to an aldose phosphate.
- i.e., from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to form fructose 6-phosphate.

- **7th Reaction**

- Erythrose 4-phosphate can also react with another molecule of xylulose 5-phosphate, to form fructose 6- phosphate and glyceraldehyde 3-phosphate.
- Enzyme is transketolase.
- Action is similar to earlier (5th) reaction. Here glycol aldehyde group is transferred from xylulose 5-phosphate to erythrose 4-phosphate, to form fructose 6- phosphate.
- Fructose 6- phosphate and glyceraldehyde 3-phosphate are the end products of non-oxidative phase of HMP shunt, which can either be converted back to glucose 6-phosphate or can be oxidised through glycolysis.
- All the reactions of the non-oxidative phase of the pentose phosphate pathway (PPP) are reversible.

Clinical Importance of HMP shunt

- In RBCs, this pathway is important to generate NADPH.



- Free radicals (super oxide - O_2^- , free hydroxy radical OH^-) and H_2O_2 generated through various oxidative processes will be scavenged by reacting with reduced glutathione (GSH). Enzyme catalysing the reaction is glutathione peroxidase.
- The oxidised glutathione (GSSG) is further reduced or the GSH is regenerated with the help of NADPH in the presence of the enzyme glutathione reductase.
- This process minimizes the oxidative stress in RBC and if there is any block in the HMP pathway, H_2O_2 and other free radicals damage the cell membrane leading to hemolysis.
- HMP is also important in RBC to maintain Fe of haemoglobin in reduced ferrous form (Fe^{2+}). Oxidized form Fe^{3+} (methemoglobin) is not able to carry oxygen.
- Methemoglobin is converted to haemoglobin by methemoglobin reductase, having NADH / NADPH as coenzyme. Methemoglobin reacts with ascorbate to form haemoglobin and dehydroascorbate. Ascorbate is regenerated in the presence of GSH catalyzed by dehydroascorbate reductase. Finally, the GSSG is reduced back to GSH by NADPH.
- RBCs of individuals with genetic deficiency of glucose 6-phosphate dehydrogenase are under oxidative stress and are prone to develop haemolytic anaemia. But such individuals are resistant to malaria since the parasite is not able to survive in RBCs under oxidative stress.
- These individuals can tolerate the oxidative stress unless exposed to certain drugs (including the antimalarial agent primaquine, aspirin or sulfonamide) or to certain herbicides, which can result in severe haemolytic anaemia. Favism is a condition arising due to the consumption of fava beans (a vegetable, which contain a toxic glycoside - divicine), leading to haemolytic jaundice, in the glucose 6-phosphate dehydrogenase deficient individuals.

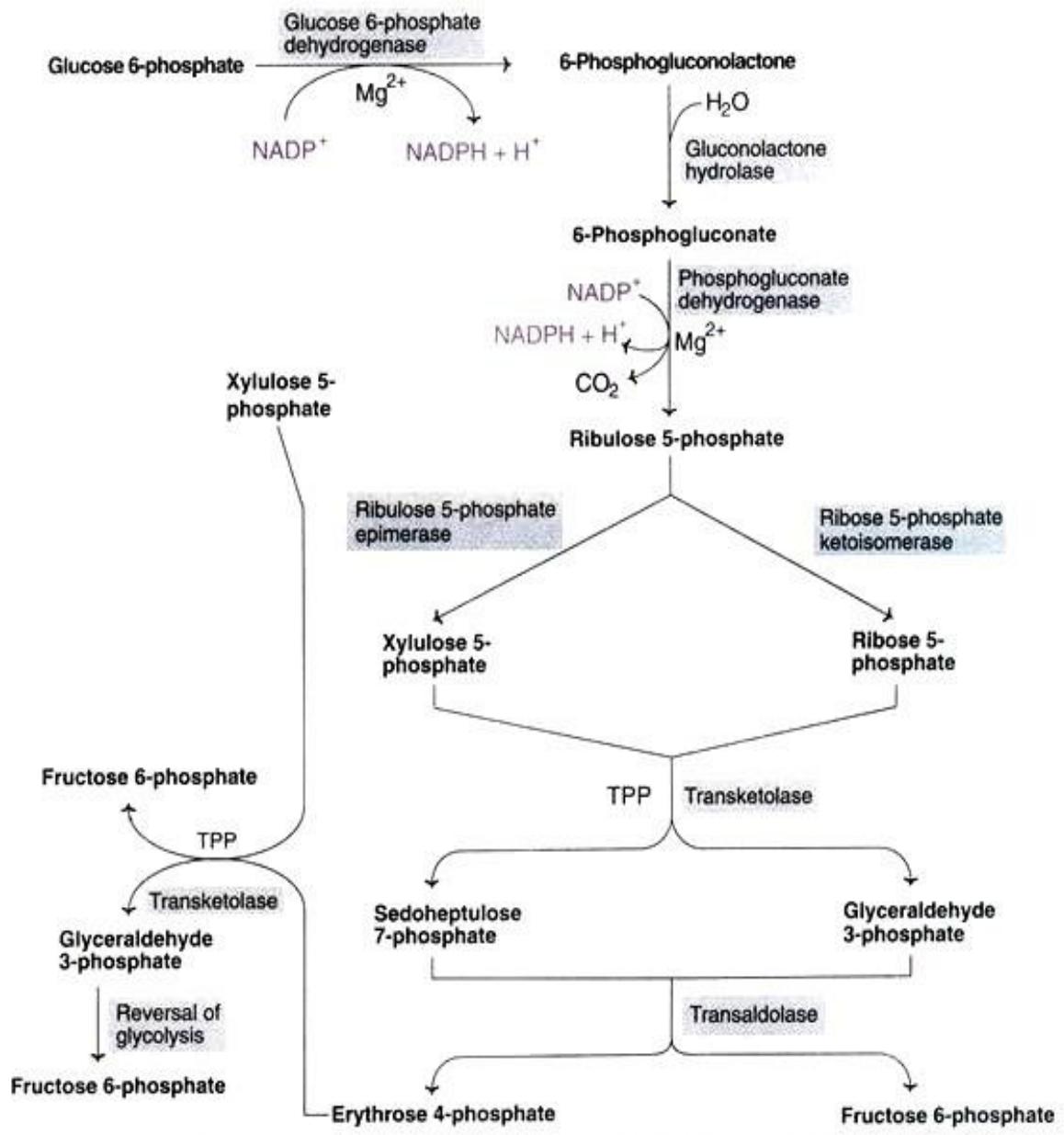


Fig: Pentose phosphate pathway / HMP pathway

Glycolytic cycle	HMP shunt
Co ₂ is not produced	Co ₂ is produced
NAD is used	NADP is used
Ribose is not formed	Ribose is formed
ATP is formed	ATP is not formed

GLYCOGENESIS, GLYCOGENOLYSIS AND REGULATION

GLYCOGENESIS

- Glycogen is a polymer of glucosyl residues having both linear chains and branches.
- Linear chains are formed by α -1, 4-glycosidic bond and at the branching point the linkage shall be α -1, 6-glycosidic bond.
- The number of branches will be more in glycogen (than starch) and there will be a branch at every 8-12 residues.
- Biological effect of branching is -
 - To make the molecule more soluble.
 - To increase the number of non-reducing ends, so that the number of sites accessible to enzymes of glycogen breakdown (glycogen phosphorylase) and glycogen synthesis (glycogen synthase) will be more.
- The synthesis of glycogen from glucose or carbohydrate sources is called as "Glycogenesis". This process occurs in cytosol.
- Glycogen is the storage form of glucose in animals. It is stored mainly in the liver and skeletal muscle as an energy source. Glycogen is synthesized when the blood glucose levels are increased.
- The advantage of storing glycogen instead of glucose is that glycogen; a polymer of glucose will have very little osmotic pressure than storing an equal amount of free glucose in cells.
- There are a number of glycogen storage diseases (genetic diseases) where glycogen cannot be degraded in a normal way. In all these cases excess glycogen will be accumulated in muscle leading to muscle weakness and the inability of the muscle cells to use glycogen as a source of energy, leading to hypoglycemia.
- Hormones regulate glycogen synthesis and degradation. An elevated insulin level results in an increase in glycogen synthesis. Insulin decreases cAMP levels.
- Increased glucagon or epinephrine causes an increase in glycogen degradation. The effects are through the increased production of cAMP.

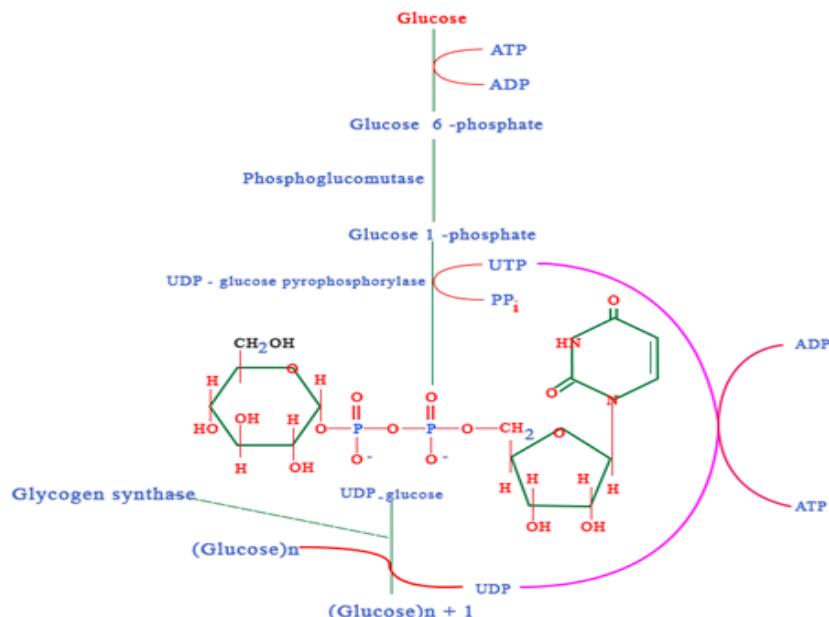
REACTIONS OF GLYCOGENESIS

- After a meal dietary glucose is absorbed from the intestine and enters the blood and then to cells.
- Glucose is first phosphorylated to glucose-6-phosphate in the presence of the enzyme, hexokinase (in muscle) / glucokinase (in liver).
- Glucose-6-phosphate then undergoes a few reactions and finally activated in the presence of UTP to UDP-glucose; then the glucose from UDP-glucose, is transferred to an existing chain of α -1,4 linked glucose ("glycogen primer"). The reactions are as follows:

- Glucose-6-phosphate is converted to glucose -1-phosphate in a reversible reaction by phosphoglucomutase.
- Glucose-1-phosphate is activated by a reaction with Uridine triphosphate (UTP) forming active nucleotide Uridine diphosphate glucose (UDPGlc). This reaction is catalyzed by UDP glucose pyrophosphorylase.
- Glycogen synthase catalyzes the addition of the glucose residues, from UDP-glucose to the non-reducing end of glycogen. This reaction is the regulatory step of glycogen synthesis. Uridine diphosphate (UDP) is then liberated. The resulting structure would be a linear unbranched molecule of glucosyl residue attached by α -1,4-glycosidic bonds.
- However initially, for transferring glucose from UDP-glucose by glycogen synthase, a primer is required which is synthesized as follows:
 - The protein "Glycogenin" has glycosyl transferase activity and is acting as the seed material for the synthesis of the glycogen primer.
 - A specific tyrosine residue is present at the 194th position of the protein glycogenin.
 - To the hydroxyl (OH) group of the tyrosine residue, the glucose molecule of UDP-glucose is transferred, forming an ester linkage, with the liberation of UDP.
 - Reaction is catalysed by the glucosyl transferase activity of the glycogenin. Here the OH of the 1st carbon of glucose will be bonded to tyrosine, leaving the 4th carbon of the glucose free.
 - Now the enzyme, glycogen synthase firmly attach with glycogenin, forming a tight complex.
 - Then seven more glucose residues will be transferred from UDP-glucose to the glucose residue attached to glycogenin, forming and α -1, 4 linked linear chains of glucose and the reaction is again catalysed by the glucosyl transferase activity of the glycogenin.
 - Once the glycogen primer with eight glucose residues is formed, further transfer of glucose will be carried out by glycogen synthase.
 - The resulting structure would be a linear unbranched molecule of glucosyl residues attached by α -1, 4-glycosidic bonds and the resulting ends will always be a non-reducing end.
- Glycogen synthase cannot make branch having α -1, 6-glycosidic bond.
- The branching enzyme (amylo-1, 4 → 1, 6-transglycosylase or glycosyl 4 → 6 transferase) catalyzes the branch formation.
- This enzyme transfers a terminal fragment of 6 to 7 glucose residues from the non-reducing end of a linear chain, having atleast 11 glucose residues to the 6th carbon of

glucose residue at another position, thus creating an α -1 \rightarrow 6 branch point. Further glucose residues will be added by glycogen synthase as α -1, 4 linkage.

- Thus the synthesis of glycogen is completed with the help of glycogen synthase and branching enzyme, and eventually, glycogen synthase detach from the complex. The glycogenin molecule remains buried within the glycogen molecule.
- Glycogen synthase exists in two forms. The active (a) form is not phosphorylated and the inactive (b) form is phosphorylated. Active form 'a' is converted to inactive 'b' form by phosphorylation by the enzymes - Protein kinases, which is activated by cAMP pathway.



GLYCOGENOLYSIS

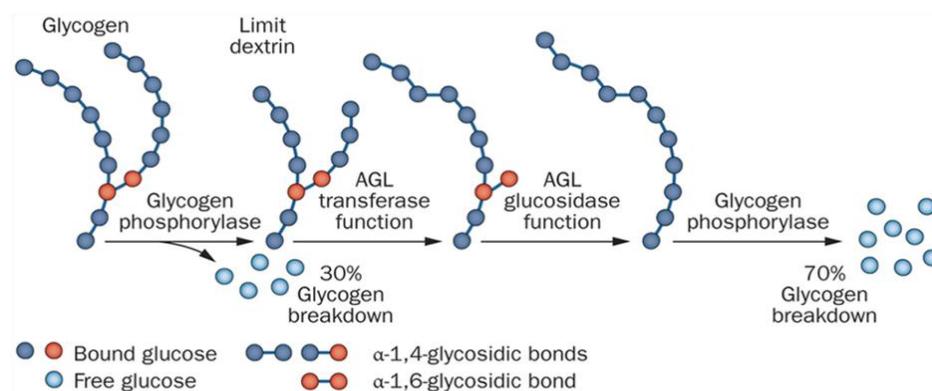
- Glycogenolysis is degradation or lysis of glycogen to glucose. Glycogen is degraded whenever blood glucose level falls.
- Glycogen phosphorylase catalyzes the sequential removal of glucose from the non-reducing end of glycogen.

REACTIONS OF GLYCOGENOLYSIS

- Glycogen phosphorylase catalyzes the sequential removal of glucose from the non-reducing end of glycogen. The glycosidic α -1, 4 bond is split with inorganic phosphate to yield glucose-1-phosphate and glycogen_{n-1}. Pyridoxal phosphate is needed as a cofactor.
- The reaction stops about four residues from a branch point is reached (α -1, 6). This results in the formation of highly branched core, which are resistant to further hydrolysis, by glycogen

phosphorylase and hence called limit dextrins. Phosphorylase cannot degrade it further. The limit dextrins are further hydrolyzed in the presence of debranching enzymes with two different activities. The reactions are as follows:

- Debranching enzyme, by its transferase activity, removes three glucose residues (which are in α -1, 4 linkage) from the four-glucosyl residues attached at the branch point, and transfers them to the non-reducing end of another chain. The elongated chain is again a substrate for glycogen phosphorylase, which releases the glucose as glucose-1-phosphate.
- The remaining single glucose attached in α -1,6 linkage at the branch point, is then removed by the hydrolase activity of the debranching enzyme, so that free glucose is released.
- Then the glucose-1-phosphate formed by phosphorylase is converted to glucose-6-phosphate by the action of phosphoglucomutase.
- In liver, the end product of glycogenolysis is glucose, formed from glucose-6-phosphate by the action of glucose-6-phosphatase.
- Muscle lacks this enzyme and is unable to release free glucose from glycogen. Hence, Glucose-6-phosphate enters glycolysis to act as fuel.
- Glycogen phosphorylase exists in two forms: The active phosphorylated form (a) and an inactive dephosphorylated form (b). The enzyme "Protein Kinase" phosphorylates the inactive form to convert it to an active form. The active form of the enzyme is converted to an inactive form by the presence of "Protein phosphatases".



HORMONAL REGULATION OF GLYCOGEN METABOLISM

- Insulin, glucagon and epinephrine are the principal hormones that control glycogen metabolism in mammals.
- Insulin is secreted when the concentration of glucose in the blood increases. Thus high levels of insulin are associated with fed state of an animal. Insulin increases the rate of glucose

transport into muscle and adipose tissue, via the glucose transporter, GLUT4. Insulin also stimulates glycogen synthesis in the liver.

- Glucagon restores the glucose concentration by stimulating glycogen degradation. Only liver cells are rich in glucagon receptors. The effect of glucagon is opposite to that of insulin and an elevated concentration is associated with the fasted / starvation state.
- The adrenal glands release the catecholamines - epinephrine and nor-epinephrine in response to neural signals. Epinephrine receptors are present both in liver and skeletal muscles. It stimulates the breakdown of glycogen to glucose 1-phosphate, which is converted to glucose 6-phosphate. The increase in intracellular glucose 6-phosphate, increases the rate of glycolysis in muscle and the amount of glucose released to the bloodstream from the liver.

GLYCOGEN STORAGE DISEASES

- They are type of genetic diseases, which reduce or prevent the release of glucose from glycogen, which lead to symptoms of hypoglycemia and excessive glycogen storage.

Von Gierke's Disease (Type I)

- Most common glycogen storage disease. This results from the deficiency of glucose 6-phosphatase. Thus, G-6-P accumulates in the liver and cannot be released as free glucose.
- G-6-P from glycogen degradation or gluconeogenesis, which normally proceeds to glucose, now has two alternate pathways.
 - Reformation of glycogen, leading to excessive accumulation of glycogen.
 - Glycolysis leading to excessive blood lactic acid.
- The inability to get free glucose from gluconeogenesis or glycogenolysis, results in hypoglycemia.

Pompe's Diseases (Type II)

- This is due to the absence of the enzyme, lysosomal α -1,6-glucosidase or acid maltase.
- Absence of this enzyme leads to the accumulation of glycogen in virtually every tissue resulting in defective lysosomal function. This disease results in massive cardiomegaly and death at an early age due to heart failure.

Cori's Disease (Type III)

- This is due to the absence of debranching enzyme. Glycogen accumulates because only the outer portion of the branches can be removed by phosphorylase.

Andersen's Diseases (Type IV)

- This is due to the deficiency of branching enzyme, which leads to the formation of very long amylopectin chains that causes the liver to become cirrhotic.

McArdle's Diseases (TYPE V)

- This is due to the absence of muscle phosphorylase.
- During exercise glycogen is not available for energy production. The individual experiences fatigue and muscle cramps.

HORMONAL REGULATION OF CARBOHYDRATE METABOLISM

INSULIN

- Insulin is a protein hormone secreted by the β -cells of pancreas.
- It is a hypoglycaemic hormone. High blood glucose level is the most important stimuli for the release of insulin.
- Insulin stimulates the glucose transporter, GLUT4, present in skeletal muscles and adipocytes, thereby increasing the transport of glucose into these tissues.
- However tissues like brain, liver, kidney cortex and blood cells do not require insulin for glucose transport; since the glucose transporter (GLUT) molecules in these tissues are different and do not require the presence of insulin for stimulation.
- Insulin indirectly helps in glucose metabolism by stimulating glucokinase and phosphorylating glucose to glucose 6-phosphate, which results in the trapping of glucose inside the cells.
- Insulin stimulates glycogen synthesis in the liver by stimulating glycogen synthase.
- Insulin promotes amino acid uptake by the cells and increases protein synthesis, thereby reducing the amino acid availability for gluconeogenesis.
- Substances like amino acids, free fatty acids, ketone bodies, glucagon, secretin and the sulfonylurea drugs, tolbutamide cause the release of insulin.
- Epinephrine and nor epinephrine inhibit the release of insulin.
- Insulin is the only hormone, which lowers blood glucose level and promotes glucose storage. cAMP levels decrease in the presence of insulin.

GLUCAGON

- A protein hormone secreted by α -cells of pancreas in response to low blood glucose level (hypoglycemia). It has its effects via the production of cAMP.
- It is a hyperglycaemic hormone.
- The effect of glucagon is opposite to that of insulin.
- Glucagon has its effect only on liver cells.
- Glucagon restores blood glucose level
 1. by stimulating glycogenolysis and
 2. by enhancing gluconeogenesis from amino acids, glycerol and lactate.

EPINEPHRINE

- It is an amino acid derived hormone, synthesised from phenyl alanine or tyrosine.
- Epinephrine is secreted from adrenal medulla in response to stressful stimuli (the 3 'F's - Fight, fright & flight, excitement, hemorrhage, hypoxia, hypoglycemia, etc.,).
- Epinephrine stimulates glycogenolysis both in liver and skeletal muscles, via the production of cAMP.
- Muscle glycogen is not a direct source of glucose due to the absence of glucose- 6-phosphatase. Therefore, glycogenolysis results in the formation of lactate, which is taken to liver for gluconeogenesis.
- Epinephrine decreases the peripheral utilization of glucose.

THYROID HORMONES - THYROXINE

- Thyroxine (T4) and Triiodo thyronine (T3) are amino acid derived hormones, synthesized from tyrosine and secreted by the thyroid gland.
- It increases the serum glucose level, which may be due to the enhanced absorption of glucose from the intestine.
- It is also involved in the depletion of glycogen. The liver, which is depleted of glycogen can be easily damaged. The damaged liver is unable to take glucose resulting in the increased glucose level.

CORTICOSTEROIDS

- Glucocorticoid hormone, cortisol is secreted from adrenal cortex.
- It increases the level of glucose in plasma mainly by gluconeogenesis in liver.
- It increases protein catabolism in extrahepatic tissues and increases amino acid uptake by the liver for gluconeogenesis.
- It also increases hepatic glycogenesis and decreases peripheral glucose utilization.

GROWTH HORMONE

- It is a peptide hormone, secreted by pituitary gland.
- It increases the blood glucose level by decreasing the glucose uptake by extrahepatic tissues and increases glucose uptake in liver.
- Growth hormone mobilizes the free fatty acids from adipose tissues, for energy production, sparing glucose, leading to an increased blood glucose level.

OXIDATION OF FATTY ACIDS

- Fatty acids are the major source of energy in most mammalian tissues and are stored as triacylglycerol or fat.
- Adipocytes are specialized cells adapted for the synthesis and degradation of triacylglycerol.
- Triacylglycerols are highly reduced compounds when compared to carbohydrates and proteins, so that oxidation of fatty acids yields more energy.
- Degradation of triacylglycerol in adipocytes is triggered by activation of hormone sensitive lipase.
- The lipase catalyzes hydrolysis of triacylglycerol to produce one molecule of glycerol and three molecules of fatty acids.
- Plants can introduce double bonds into fatty acids in the region between C10 and the ω end so that they can synthesize ω 3 and ω 6 fatty acids.
- Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C9 in the fatty acid chain; thus they cannot synthesize linoleate and linolenate, which thus become essential fatty acids to be present in the diet.
- Oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because they are having less H atom (more double bonds). Hence, fewer reducing equivalents can be produced.
- When an unsaturated fatty acid undergoes β -oxidation, an NADPH dependant reduction is required for each double bond in the fatty acid. This reduces the yield of ATP by 2.5 for each double bond when compared to the corresponding saturated fatty acid.

BETA-OXIDATION OF FATTY ACIDS

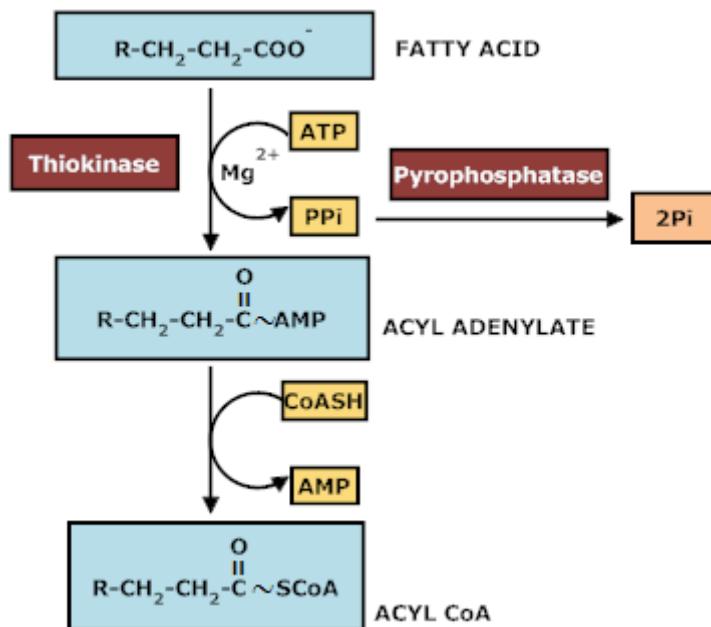
- This pathway was elucidated by Knoop (1905).
- Oxidation occurs between the α and the β carbon atoms of the fatty acid, and hence it is called as “ β -oxidation”, which degrades fatty acids starting at the carboxyl end to acetyl-CoA units.
- The acetyl-CoA produced, enters the citric acid cycle for the production of energy (or) may be used for the synthesis of ketone bodies. Both citric acid cycle and β -oxidation occurs within the mitochondrial matrix and yields the reduced nucleotides FADH_2 and NADH .
- The reducing equivalents from this electron – carriers are fed into electron transport chain to produce ATP. Cells lacking mitochondria cannot oxidize fatty acids.

Reactions of beta - oxidation

- The oxidation of fatty acids, occurs in 3 stages,
 - Activation of fatty acids, in the cytoplasm.
 - Transfer of activated fatty acids into the mitochondrial matrix.
 - Oxidation of fatty acids to yield acetyl-CoA.

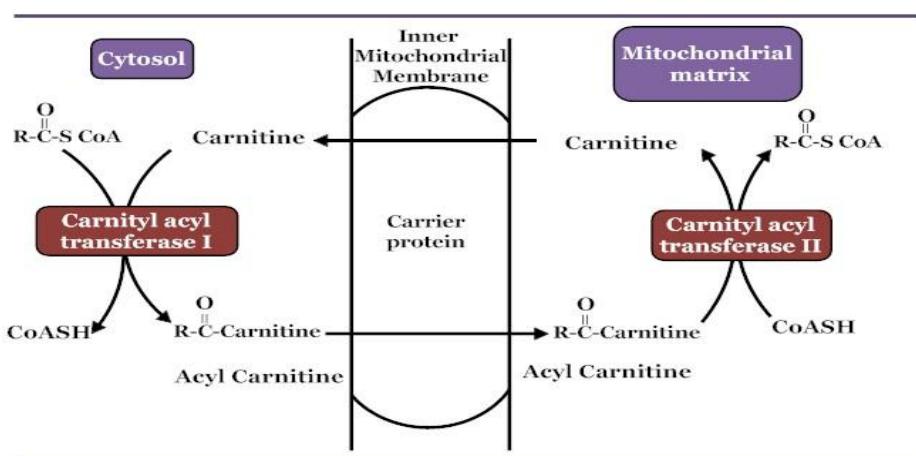
Activation of fatty acids

- The free fatty acids in the blood diffuse into the cytoplasm.
- In cytoplasm, fatty acids are activated by the enzyme called “thiokinase” or “acyl-CoA synthetase” (found in the outer membrane of mitochondria) in the presence of ATP and CoA, to form fatty acyl CoA, AMP and PPi.



Transfer of activated fatty acids into the mitochondrial matrix

- Short chain fatty acids could cross the inner mitochondrial membrane easily, without the help of any transporter.
- Long chain fatty acids or their acyl-CoA form cannot cross the inner mitochondrial membrane.
- They are transported across the membrane with the help of a transporter called “carnitine transporter” (β -hydroxy- γ -trimethyl ammonium butyrate).



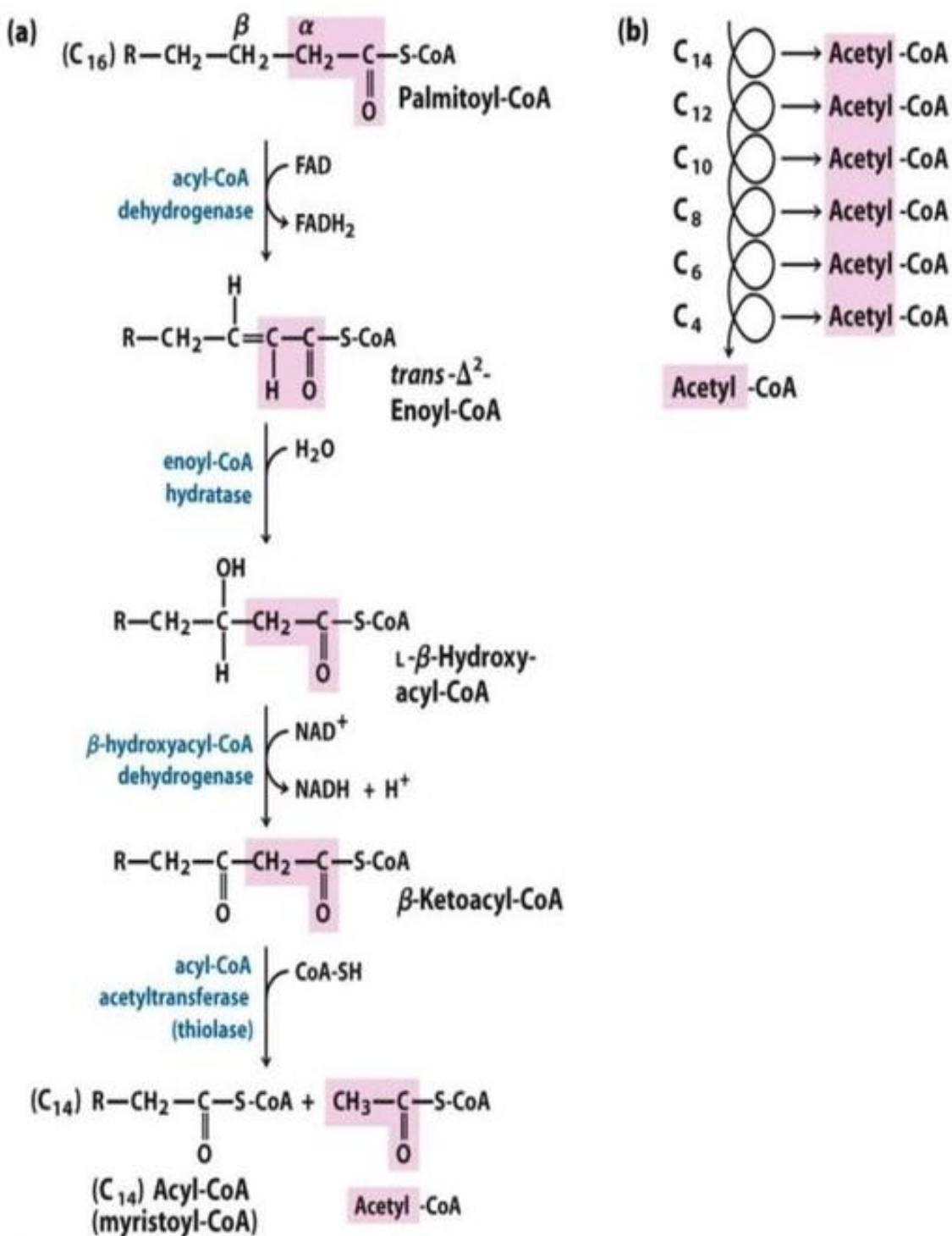


Fig: β -oxidation pathway of fatty acids (Palmitic acid)

(a) In each pass through this sequence, one acetyl residue (shaded in red) is removed in the form of acetyl-CoA from the carboxyl end of palmitate (C₁₆), which enters as palmitoyl-CoA. (b) Six more passes through the pathway yield seven more molecules of acetyl-CoA, the seventh arising from the last two carbon atoms of the 16-carbon chain. Eight molecules of acetyl-CoA are formed in all.

Oxidation of fatty acids

- **1st reaction - Oxidation at α and β carbons**
 - One hydrogen each, from the α and the β carbons were removed in the presence of the enzyme acyl- Co A dehydrogenase to form α,β unsaturated fatty acyl CoA i.e. Delta-2 trans enoyl CoA.
 - Coenzyme is FAD, which is converted to FADH₂, and enters ETC, producing 1.5 ATP.
- **2nd reaction - Hydroxylation at the double bond**
 - A molecule of water is added across the double bond forming β -hydroxy acyl CoA (or 3 hydroxy acyl-Co A) in the presence of the enzyme enoyl CoA hydratase.
- **3rd reaction - Oxidation of β carbon**
 - Two hydrogens from the β carbon is removed in the presence of the enzyme hydroxyacyl-Co A dehydrogenase, to form β -keto acyl CoA.
 - Coenzyme is NAD⁺ which is converted to NADH and enters ETC to produce 2.5 ATP.
- **4th reaction - Cleavage of β (3) -keto acyl CoA**
 - The β -keto (3 keto) acyl-Co A then undergoes a cleavage catalyzed by the enzyme ketothiolase in the presence of another Co A yielding a fatty acyl-CoA and acetyl-CoA.
 - The fatty acyl CoA formed is 2-Carbon atom shorter than the original fatty acyl CoA.
 - Once started, this process continues until all the fatty acid is converted to acetyl Co A.
 - Oxidation of fatty acids with an odd number of carbon atoms produces acetyl CoA and one molecule of Propionyl – Co A as final products.
 - This Propionyl-Co A is converted into Succinyl-Co A and enters TCA cycle, which can be oxidised or utilized for gluconeogenesis.

SIGNIFICANCE OF BETA OXIDATION

- High carbohydrate diet suppresses fatty acid oxidation. Peroxisomes also carry out β -oxidation. Hypoglycemic drugs like sulfonyl urea (glibenclamide and tolbutamide) reduce fatty acid oxidation by inhibiting carnitine palmitoyl transferase (carnitine transporter).
- Gluconeogenesis is dependent upon the fatty acid oxidation. Any impairment in fatty acid oxidation, leads to hypoglycemia. Increased fatty acid oxidation, characteristic of starvation and diabetes mellitus, leads to ketone body production by the liver (ketosis).
- Hypoglycin, a plant toxin inhibits β -oxidation, by inactivating acyl-Co A dehydrogease, which leads to hypoglycaemia.

Energy yield from beta - Oxidation

- The energy yield from the beta -oxidation pathway is high.
- For example, the oxidation of a molecule of palmitoyl-Co A (which contains 16 carbon units) to CO₂ and H₂O yields the following number of ATPs.
- Beta oxidation of palmitoyl CoA yields 7 FADH₂, 7 NADH and 8 acetyl CoA.

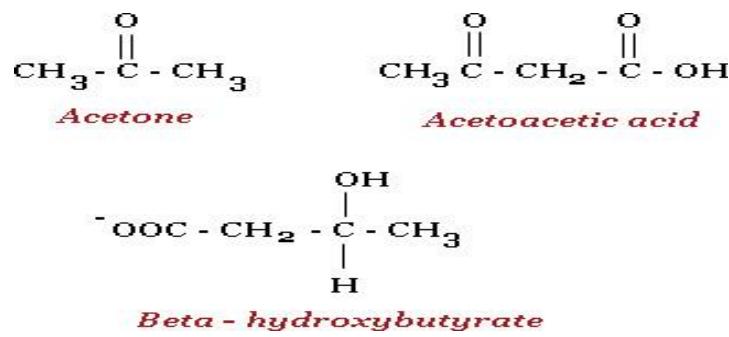
S. No.	Energetics of Palmitoyl – Co A	ATP Yield	
		New	Old
1.	7 FADH ₂ , which provide 1.5 ATP when oxidized by the respiratory chain	7 x 1.5 = 10.5	7 x 2 = 14
2.	7 NADH, which, each provides 2.5 ATP when oxidized by the respiratory chain	7 x 2.5 = 17.5	7 x 3 = 21
3.	Each acetyl-Co A provides 10 ATP when Converted to CO ₂ and H ₂ O by the TCA Cycle. Thus, 8 acetyl-Co A yields	8 x 10 = 80.0	8 x 12 = 96
	Total ATP yield	108	131
ATP Utilization			
4.	To activate the fatty acid in the first step ATP is converted to AMP and PP _i , utilizing two high energy bonds, which is equivalent to 2 ATP	2	2
	Net ATP yield	106	129

OTHER TYPES OF OXIDATION OF FATTY ACIDS

- Unusual fatty acids may be oxidized by α -oxidation and ω -oxidation, which are minor pathways.
- α -oxidation occurs in brain especially in the endoplasmic reticulum. This is involved in the metabolism of methylated fatty acids. First hydroxylation occurs at α - carbon followed by oxidation and decarboxylation releasing CO₂ from the carboxyl terminus, providing the substrate that can be metabolized through β -oxidation. This type of oxidation does not generate high-energy compounds.
- ω -oxidation occurs in endoplasmic reticulum, which involves hydroxylation and oxidation at the terminal methyl group of fatty acids to form a dicarboxylic acid, which undergoes β - oxidation; this type of oxidation occurs in peroxisome also. The end product may be excreted in the urine.
- Oxidation of unsaturated fatty acids requires two additional enzymes
 - Enoyl-CoA isomerase, which converts cis-isomer to a trans-isomer
 - 2, 4-dienoyl-CoA reductase, which is involved in the addition of hydrogen atoms across the double bonds.

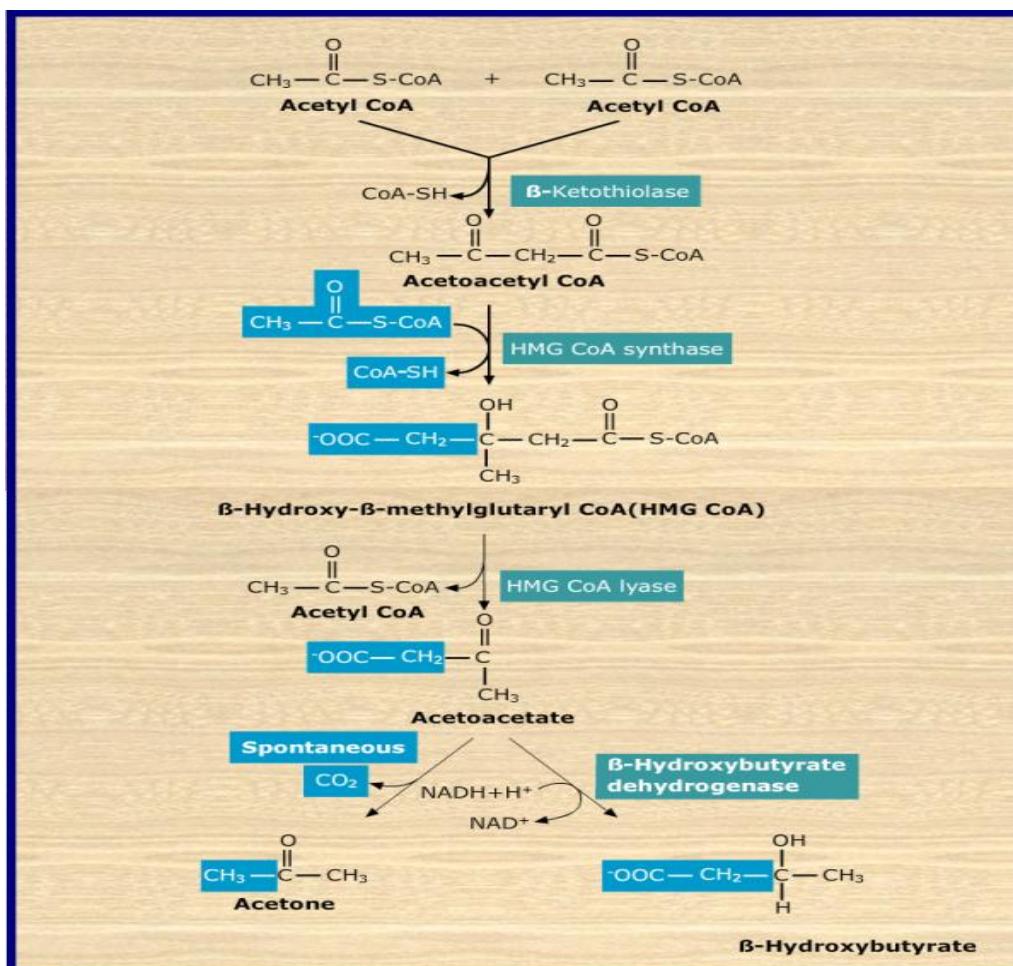
KETONE BODIES

- Acetone, Acetoacetate and β -hydroxybutyrate are considered as ketone bodies. They are important sources of energy for the peripheral tissues.
- The ketone bodies are water-soluble compounds, produced mainly in liver mitochondrial matrix. Liver has the capacity to divert any excess acetyl-CoA derived from fatty acid, pyruvate oxidation or break down of amino acids into ketone bodies.
- The entry of acetyl-Co A into citric acid cycle is determined by the availability of oxaloacetate.
- As ketone bodies are soluble in water, they could be transported through blood without the involvement of any transporters.
- Excess amount of ketone bodies are produced in starvation and diabetes mellitus.
- In starvation, oxaloacetate is drawn out of the citric acid cycle for the synthesis of glucose, thereby decreasing the availability of oxaloacetate, resulting in the accumulation of acetyl-Co A favouring the formation of ketone bodies.
- In diabetes mellitus, due to the deficiency of insulin, the available glucose is not transported to cells of skeletal muscles and adipocytes. So, to obtain energy, the cell depends on the β -oxidation of fatty acids, resulting in the overproduction of acetyl-CoA, leading to the synthesis of ketone bodies.
- The presence of excessive amount of ketone bodies in blood is called as “ketonemia”, and it results in the appearance of ketone bodies in urine called as ““ketonuria” and in milk (ketolactia).
- Excess ketone bodies in the blood, decreases blood pH, resulting in “ketoacidosis”.
- As ketone bodies complex with Na^+ and K^+ , urinary loss of ketone bodies result in loss of electrolytes and polyuria.
- Under starvation, ketone bodies replace glucose as a major fuel in the central nervous system.
- In non-ruminants, liver is the sole source of ketone body production. In ruminants, rumen epithelium and mammary glands are also the sources of ketone bodies.



KETOGENESIS

- Ketone body synthesis or Ketogenesis - 3 steps:
 - First two molecules of acetyl-CoA condense to form acetoacetyl-CoA in a reaction catalyzed by thiolase.
 - Subsequently, a third molecule of acetyl - CoA is added to acetoacetyl - CoA to form 3-hydroxy-3-methylglutaryl -CoA (HMG -CoA) in a reaction catalyzed by HMG-CoA synthetase. These steps are same as cholesterol synthesis.
 - The HMG - CoA is cleaved by HMG - CoA lyase producing acetoacetate and acetyl-CoA.
 - Acetoacetate can be converted to :
 - β - hydroxybutyrate, by reduction in the presence of NADH catalysed by the enzyme β - hydroxybutyrate dehydrogenase and
 - Acetone by a non-enzymatic decarboxylation reaction.
 - The carboxyl group of ketone body has a pK_a around 4. Hence, each ketone body loses a proton, which lowers blood p^H .



KETOLYSIS

Oxidation of ketone bodies

- The liver lacks the enzyme called “*thiophorase*” and therefore cannot use the ketone bodies as fuel.
- However, extra-hepatic tissues such as heart, skeletal muscles can utilize them as fuels.
- β - hydroxybutyrate is converted to acetoacetate in a reaction catalyzed by β - hydroxybutyrate dehydrogenase producing NADH.
- Acetoacetate receives CoA from Succinyl-CoA to form acetoacetyl-CoA in a reaction catalyzed by Succinyl-CoA: acetoacetyl-CoA transferase (also known as thiophorase).
- Thiolase catalyzes the conversion of acetoacetyl-CoA to two molecules of acetyl-CoA, which can be oxidized by the citric acid cycle.

FATTY ACID BIOSYNTHESIS

- Fatty acids are synthesized when there is excess nutrient availability (glucose). These will be esterified with glycerol and stored as triacylglycerol or the fatty acids will also used for the synthesis of phospholipids to form cell membrane.
- Fatty acid synthesis occurs mainly in the *liver and adipocytes* and to a lesser extent in mammary gland during lactation.
- The synthesis takes place in *cytosol*.
- Acetyl CoA and NADPH are the two important prerequisites for fatty acid synthesis along with ATP.
- NADPH supplies the hydrogen atoms required for the synthesis. The HMP shunt is the major source of NADPH. The other source is by the action of malic enzyme.
- The overall synthesis can be divided into 3 steps
 - Production of acetyl CoA and NADPH
 - Formation of malonyl Co A from acetyl Co A
 - Reactions of Fatty acid synthase complex

STEP I: PRODUCTION OF ACETYL CoA AND NADPH

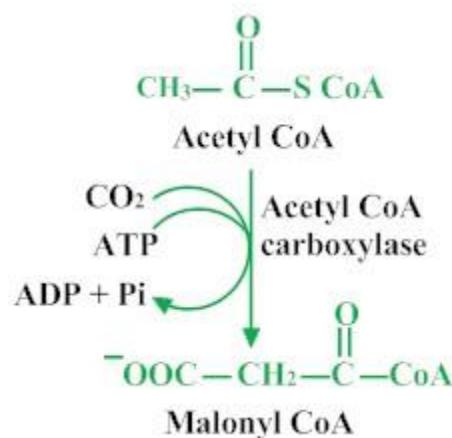
- Acetyl-CoA is the starting material for the synthesis of fatty acids. Most of the acetyl-CoA is derived from carbohydrates and to a lesser extent from amino acids.
- The pyruvate formed in glycolysis is transferred to mitochondria, where it is converted to acetyl- CoA by the enzyme pyruvate dehydrogenase complex. The CoA derivatives are unable to cross the inner mitochondrial membrane. Hence, acetyl-CoA is transferred from

mitochondria to cytosol in the form of citrate by condensing with oxaloacetate. This reaction is catalyzed by the enzyme citrate synthase.

- In the cytosol, citrate is cleaved to form oxaloacetate and acetyl-CoA in an ATP dependent reaction catalyzed by citrate lyase (citrate cleavage enzyme or malic enzyme).
- There is little citrate lyase or malic enzyme in ruminants because in this species, acetate (derived from carbohydrate digestion in the rumen and activated to acetyl-CoA, extra mitochondrially) is the main source of acetyl CoA.
- NADPH is obtained in two ways:
 1. By converting cytosolic oxaloacetate to malate by malate dehydrogenase and subsequent oxidative decarboxylation of malate to pyruvate and CO_2 with the production of NADPH, in the presence of malic enzyme.
 2. Through HMP shunt.

STEP II : FORMATION OF MALONYL CoA FROM ACETYL CoA

- The acetyl-CoA is carboxylated to form malonyl-CoA, catalyzed by acetyl-CoA carboxylase, a biotin dependent enzyme. This enzyme is the regulatory enzyme in the synthesis of fatty acids.



STEP III: FATTY ACID SYNTHASE COMPLEX

- Fatty acid synthase is a multienzyme complex possessing 7 different enzyme activities.
- The fatty acid synthase of mammals is a dimer composed of two identical subunits. The complex has two thiol groups. One is the $-SH$ group of cysteine residue and the other is the $-SH$ group of ACP (acyl carrier protein) containing 4-phosphopantetheine.
- To initiate biosynthesis, acetyl group is first attached to the $-SH$ group of phosphopantetheine catalyzed by acetyl transacylase and then shifted to sulfhydryl group of cysteine. Malonyl group of malonyl-CoA is transferred to $-SH$ group of phosphopantetheine of ACP to form acetyl-malonyl enzyme.
- The acetyl group attacks the methylene group of the malonyl-CoA to form 3-ketoacyl enzyme, which is now attached to $-SH$ group of phosphopantetheine. This reaction is catalyzed by 3-

ketoacyl synthase. One molecule of CO₂ is released, which acts as a driving force for the whole sequence of the reaction. Note that the –SH group of cysteine is now free.

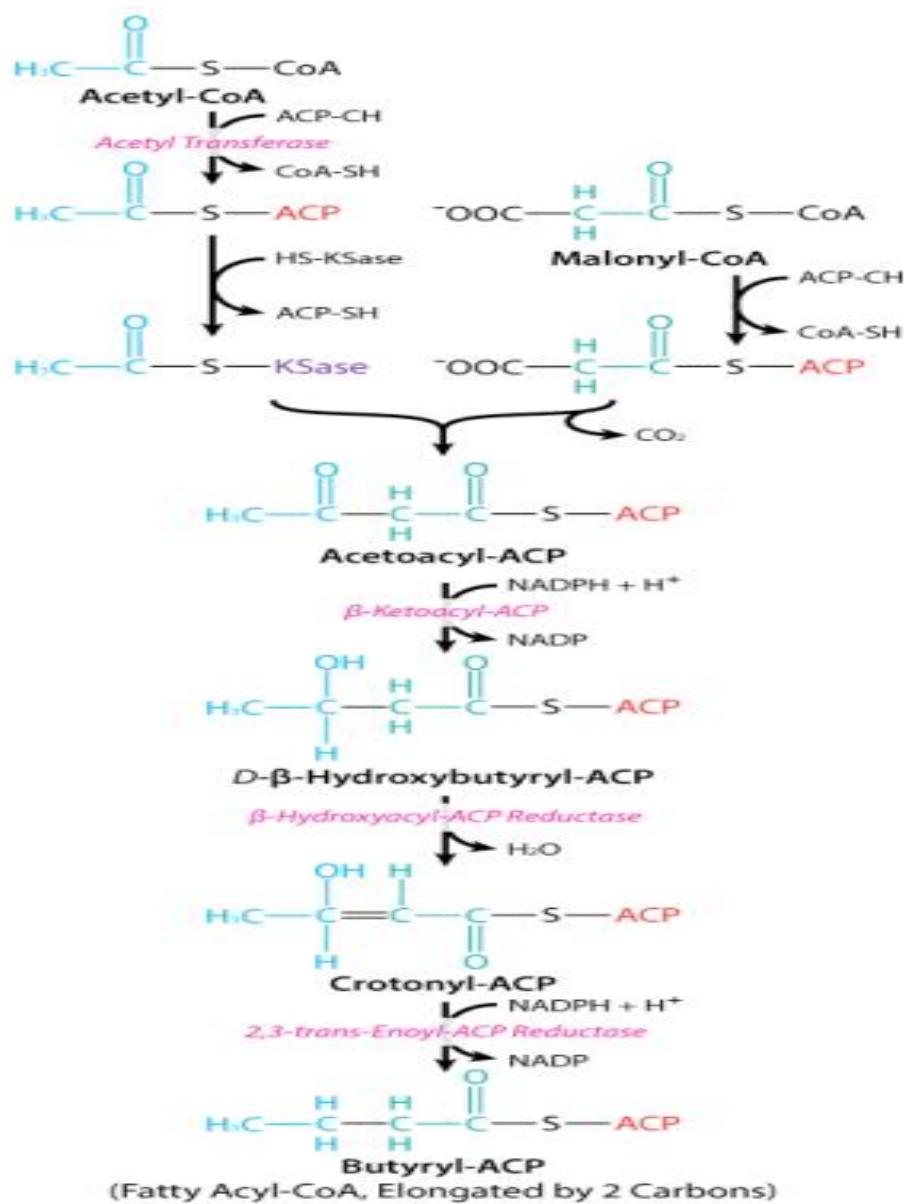
- The 3-ketoacyl group is reduced to 3-hydroxyacyl enzyme at the expense of NADPH in a reaction mediated by 3-ketoacyl-ACP reductase. The next step involves a dehydration to form a double bond (2,3trans-enoyl-CoA) by the enzyme 3-hydroxyacyl CoA dehydratase. The double bond is then reduced by NADPH in a reaction catalyzed by 2,3-transenoyl-CoA reductase. Two NADPH molecules are needed for every addition of two carbon unit that is added during biosynthesis. The net result is that the original acetyl group is elongated by 2 carbons.
- The fatty acyl chain is then transferred to the –SH group of cysteine. A new malonyl CoA attaches to the –SH group of phosphopantetheine. The fatty acyl moiety condenses with malonyl moiety with the release of CO₂. The reduction, dehydration and reduction are repeated until the chain is 16 carbon in length. At this point hydrolysis occurs and the palmitate is released.
- The palmitate is elongated and desaturated to produce a series of fatty acids. The newly formed fatty acids are used for the synthesis of triacylglycerol, which are then incorporated into VLDL for export.
- The acetyl CoA involved in the first reaction becomes the penultimate and terminal carbons of the growing chain. For the first reaction alone acetyl CoA is required. In the subsequent reactions the two carbon units are contributed by malonyl-CoA.

BIOENERGETICS OF FATTY ACID SYNTHESIS

For the synthesis of one molecule of palmitate

Reactions	ATP utilized
1 ATP / molecule of acetyl CoA from citrate. Therefore, for 8 molecules of acetyl CoA (to form one molecule of palmitate)	8
1 ATP / molecule malonyl CoA. Therefore for 7 malonyl CoA	7
Total ATP utilized	15

- In addition 14 molecules of NADPH are needed to synthesize one molecule of palmitate from citrate.



ELONGATION AND DESATURATION OF FATTY ACID SYNTHESIS

Elongation

- The end product of the fatty acid synthesis is palmitic acid.
- In cells fatty acids having chain length of C18, C20,C22 and C24 are commonly seen.
- The synthesis of these fatty acids occurs mainly in the endoplasmic reticulum using malonyl CoA and NADPH.
- The palmitate participates as CoA derivative instead of fatty acyl ACP.

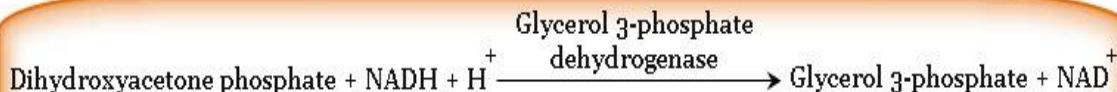
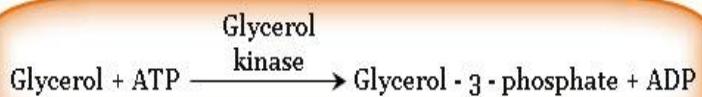
Desaturation

- Fatty acid synthase catalyzes the formation of palmitate, a saturated fatty acid.
- Human cells possess enzymes to convert saturated to unsaturated fatty acids. The desaturation process can occur at C9 position or position closer to the carboxyl group.
- The enzyme is Δ^9 desaturase, present in the endoplasmic reticulum, that catalyses the conversion of palmitoyl CoA or stearoyl CoA to palmitoleyl CoA or oleoyl CoA respectively. Oxygen and either NADH or NADPH is necessary for the reaction.
- Humans and many animals have no enzyme that can introduce double bonds beyond carbon 9, so linoleic (18:2 $\Delta^{9,12}$) and linolenic (18:3 $\Delta^{9,12,15}$) acids cannot be synthesized. Hence, these two unsaturated fatty acids are nutritionally essential fatty acids, to be supplied in the diet.
- In most mammals arachidonic acid and eicosapentaenoic acid are produced in the liver from linoleic and linolenic acid respectively.
- Conversion of linoleate to arachidonate is catalysed by Δ^6 desaturase activity, followed by the microsomal chain elongation system (Elongase) and lastly the Δ^5 desaturase action.
- Cats cannot carry out this conversion due to the absence of Δ^6 desaturase activity.
- However, plants can introduce double bonds into fatty acids in the region between C10 and the ω end so they can synthesize ω 3 and ω 6 fatty acids i.e. all essential fatty acids.

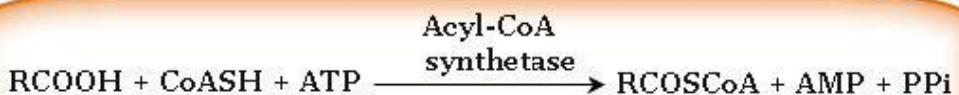
BIOSYNTHESIS OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS

BIOSYNTHESIS OF TRIACYLGLYCEROL

- Synthesis of TAG occurs in adipose tissue and liver. Smooth endoplasmic reticulum is the site of synthesis
- Action of lipoprotein lipase on lipoproteins release free fatty acids, which are transported into the adipose tissue, where the fatty acids are converted to triacylglycerol for storage.
- Glycerol is the backbone in the synthesis of triacylglycerol and some phospholipids.
- Glycerol 3-phosphate is the substrate for triacylglycerol, which can be synthesized directly from glycerol by the enzyme glycerol kinase present in the liver, lactating mammary gland and brown adipose tissue. In muscle and adipose tissue, glycerol kinase is absent or low in activity.
- Glycerol 3-phosphate is also formed from glycolysis from glucose (dihydroxyacetonephosphate is reduced to glycerol 3-phosphate in a reaction catalyzed by glycerol 3-phosphate dehydrogenase).



- For the attachment of fatty acid in ester bond to glycerol, the acid must be activated to acyl-CoA, a reaction catalyzed by acyl-CoA synthetase.



- Two molecules of acyl-CoA are added to the free hydroxyl group of glycerol 3-phosphate, first at C₁ to produce monoacylglycerol 3-phosphate and then to C₂ to form diacylglycerol 3-phosphate (phosphatidic acid). The phosphate is removed by a phosphatase and the third acyl group is added to form triacylglycerol.

MOBILIZATION OF FATTY ACIDS

- Mobilization of fatty acids involves the release of free fatty acids and glycerol from triacylglycerol by lipase. The adipose tissue lipase is stimulated by epinephrine, glucagon and ACTH through the II messenger cAMP. Insulin inhibits the activity of lipase.
- The released glycerol is converted to dihydroxyacetone phosphate and enters the glycolytic cycle in the liver.
- All long chain fatty acids must bind to protein for their transport to other parts. Albumin is the transport protein in plasma.

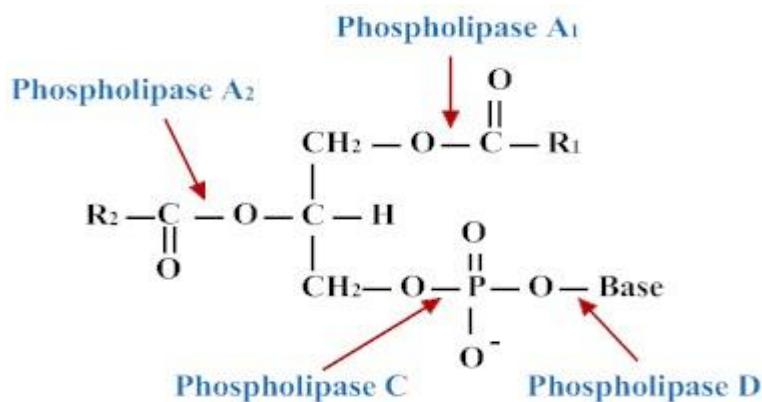
BIOSYNTHESIS OF PHOPHOLIPIDS

- Phosphatidic acid is the precursor for the synthesis of various phospholipids.
- The compound that may bind to the phosphoric acid group of phosphatidic acid may be choline, ethanolamine, serine, inositol, or glycerol, resulting in different phospholipids.
- Generally the fatty acid at C 1 is saturated and that at C 2 is unsaturated.
- The general outline for synthesis of the above phospholipids are depicted below in two animations.
- For example, phosphatidylcholine is synthesised as follows:

- The process requires the presence of diacylglycerol and an activated choline.
- Choline is activated first by phosphorylating with ATP to form phosphocholine, catalyzed by choline kinase.
- Next phosphocholine reacts with CTP to form CDP-choline, which is catalyzed by phosphocholinecitidyltransferase.
- Finally the CDP-choline reacts with diacylglycerol to form phosphatidylcholine and CMP, catalyzed by phosphocholine-diacylglycerol transferase.
- Similar kind of reactions result in the synthesis of other phospholipids.
- In mammals, phosphatidyl serine is not synthesised from CDP-diacyl glycerol, instead it is derived from phosphatidyl ethanolamine via the head-group exchange reaction.
- The above animation depicts the flow chart for the synthesis of phosphatidyl choline (Lecithin), phosphatidyl ethanolamine (Cephalin) and phosphatidyl serine.
- The following animation depicts the flow chart for the synthesis of phosphatidyl inositol, phosphatidyl glycerol and cardiolipins, which again start from phosphatidic acid that gets converted into CDP-diacylglycerol.

ACTION OF PHOSPHOLIPASE

- A_1 removes the acyl group from carbon 1 of phospholipid.
- A_2 also releases acyl group from carbon 2 of phospholipid.
- B acts on the lysophospholipid, after the action of A_2 .
- C liberates diacylglycerol from phospholipids.
- D produces phosphatidic acid from various phospholipids.



LIPOPROTEIN METABOLISM

CLASSIFICATION OF LIPOPROTEINS

- Fat like triacylglycerol, phospholipids and cholesterol, which are absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between various tissues and organs for utilization and storage.
- Since lipids are insoluble in water, it is difficult to transport them in an aqueous medium – the plasma. This is solved by associating non-polar lipids (triacylglycerol and cholesteryl ester) with amphipathic lipids (phospholipids and free cholesterol) and protein to make water-soluble miscible lipoproteins.
- Lipoproteins are transported in the plasma as lipoproteins. Protein present in lipoprotein increases the solubility of lipid.
- Lipoproteins can be separated and identified by several methods including electrophoresis and ultracentrifugation (separation by density).
- Based on the density, lipoproteins have been classified into four major groups. As pure fat is less dense than water, when the lipid portion is more than the protein in a lipoprotein the density of the lipoprotein is decreased.
 - Chylomicrons, derived from intestinal absorption of triacylglycerol.
 - Very low-density lipoprotein (VLDL) derived from the liver for the export of triacylglycerol
 - Low-density lipoprotein (LDL). It represents the final stage in the catabolism of VLDL.
 - High density lipoprotein (HDL). Involved in chylomicron and VLDL metabolism and also cholesterol transport.
- Triacylglycerol is the predominant lipid in chylomicron and VLDL. Whereas cholesterol and phospholipid are the predominant lipid in LDL and HDL respectively.

ELECTROPHORETIC SEPARATION AND STRUCTURE OF LIPOPROTEIN

- Plasma lipoprotein analyses are made routinely in clinical lab by electrophoresis on cellulose acetate. Four major bands are obtained when plasma lipoproteins are separated by electrophoresis and stained by oil 'O' red.
- One band remains at the origin, which contains chylomicron. The others are called α and the β lipoproteins.
- The second band contains β lipoproteins, which migrate with the β globulins and contain LDL.
- The third band migrates in front of the β lipoprotein and contains VLDL.
- The fourth band, called α - lipoprotein, migrates with the α - globulins and contains HDL.

Structure of a lipoprotein

- A lipoprotein such as chylomicron or VLDL consists of mainly non-polar lipids like triacylglycerol and cholesteryl ester at the center, which are surrounded by a single surface layer of amphipathic phospholipid and free cholesterol molecules, with their polar groups facing outwards to the aqueous medium.
- The protein moiety of the lipoprotein is known as apolipoprotein or apoprotein.
- Some apolipoproteins are integral and cannot be removed. Whereas others are free to transfer to other lipoproteins.
- One or more apolipoproteins are present in each lipoprotein.
- Apolipoprotein of HDL is known as 'A', while that of VLDL and chylomicron is B.
- Apolipoprotein CI, CII and CIII are freely transferable between different lipoproteins.
- The apolipoprotein E has been isolated from VLDL and HDL.
- Apoprotein found in domestic animals are A-I,AII,A-IV, B48,B100, C-I,C-II,C-III,CIV and E

FUNCTIONS OF APOLIPOPROTEIN

- They are the components of the structure of lipoproteins. In chylomicron, the apoprotein is apoB₄₈, in VLDL, it is apoB₁₀₀
- Some apoproteins are required to activate certain enzymes for the metabolism of lipoproteins. For example apo CII is necessary for the activation of lipoprotein lipase, which acts on chylomicrons and AI for activation of LCAT.
- They can bind to specific receptors on the surface of the cells in tissues, eg. Apo B 100 and apo E for the LDL receptor. Apo AI for the HDL receptor. In this way individual lipoproteins are taken up only by the concerned cells.
- They can act as lipid transfer protein. E.g. Apo-D in HDL.

METABOLISM OF CHYLOMICRONS

- The chylomicrons are the lipoproteins with lowest density and largest size.
- They transport all dietary lipids like triacylglycerol and cholesteryl ester into circulation via the lymph.
- The major apolipoprotein is B 48. When it reaches the plasma; chylomicron is rapidly modified by receiving apo E and apo CII from HDL.
- Apo E in conjugation with B-48 is recognized by hepatic receptors. The apo CII is necessary for the activation of lipoprotein lipase, the enzyme present on the capillary walls of most tissues.
- Lipoprotein lipase activated by apo CII hydrolyzes the triacylglycerol of chylomicron to yield monoacylglycerol, fatty acid and glycerol.
- Heart lipoprotein lipase has a low Km for triacylglycerol, whereas Km of the enzyme in adipose tissue is 10 times greater.

- As the chylomicron circulates, the triacylglycerol is degraded and the size is reduced resulting in an increase of density. The apoprotein CII are returned to HDL. The remaining particle is called remnant.
- In humans the remnants are removed from the circulation by the liver. The hepatocyte membranes contain lipoprotein receptors that recognize the combination apoprotein B-48 and E. The remnants bind to these receptors and are taken into the cell by endocytosis.
- The endocytosed vesicles then fuse with the lysosome and apolipoprotein, cholestryl ester are degraded releasing amino acid and free cholesterol and fatty acids. The free cholesterol inhibits cholesterol synthesis in the liver by decreasing the concentration of HMG-CoA reductase and also by inhibiting the action of the enzyme.

METABOLISM OF VLDL

- VLDL is produced in the liver. The main function of VLDL is to carry the lipids synthesized in the liver to the peripheral tissues.
- The liver produces triacylglycerol. They are packaged along with cholesterol, phospholipids and apo B-100 and are secreted into blood as VLDL. The triacylglycerol is degraded by lipoprotein lipase as in chylomicrons.
- In the blood HDL transfers apo CII, apo-E as well as cholestryl esters. When the TG is removed, size of VLDL decreases and becomes denser. The apo CII and apo E, then return to HDL.

METABOLISM OF LDL

- After the above modifications, VLDL is converted to plasma LDL. It retains apo-B 100 and much less TG than VLDL and has high concentration of cholestryl ester.
- The primary function of LDL is to provide cholesterol to the peripheral tissues.
- The receptor on the cell surface membrane of the liver or extra hepatic tissues recognizes apolipoprotein B-100 of LDL, which is taken up by the process of endocytosis. It is then fused with lysosome for degradation.
- In humans, 80% of the cholesterol carried in the blood is as LDL. Increased LDL is correlated to heart disease. Hence, LDL is called as bad cholesterol. Whereas in animals there are species variations in the cholesterol transport.

METABOLISM OF HDL

- HDL is synthesized mainly in the liver and also from the gut. HDL from gut does not contain apo C or E, but only apo A. Apo C and E are synthesised in the liver and transferred to gut HDL, when they enter the plasma. It is the source of apolipoprotein needed for the metabolism of other lipoproteins. It transfers apo CII and apo E to VLDL and chylomicron, the TG rich lipoproteins.
- Apo CII is an activator of lipoprotein lipase, which degrades triacylglycerol. HDL also exchanges lipids and protein with chylomicron and VLDL. It picks up free cholesterol from the surface of

the cells and from other lipoproteins and converts it to cholestryl esters, which is catalyzed by the enzyme LCAT (Lecithin-cholesterol acyltransferase).

- LCAT catalyses the transfer of fatty acid residue from 2nd position of lecithin to cholesterol to form cholestryl ester.
- Some cholestryl ester is transferred to VLDL in exchange for TG and remain in LDL. This exchange is mediated by plasma lipid transfer protein. The LDL is taken up by liver and the cholestryl ester is metabolized. The transfer of cholesterol from extrahepatic tissues to liver is called reverse cholesterol transport.
- The HDL is finally returned to liver, where cholestryl ester is degraded. The cholesterol released may be repackaged in lipoprotein or catabolized to bile acids
- In humans, high levels of HDL have been associated with decreased risk of heart disease. Because cholestryl esters of HDL are ultimately returned to liver and are mostly converted to bile acids and excreted. Hence, HDL is called as good cholesterol. There are 3 types of HDL designated as HDL₁, HDL₂ and HDL₃. HDL₁ has more cholesterol than other two HDLs. HDL₂ concentration is inversely related to the incidence of coronary atherosclerosis.

HEART DISEASES AND SPECIES VARIATION

Cholesterol and heart diseases

- Incidence of cardiovascular disease is correlated with the concentration of plasma cholesterol.
- Individuals with elevated levels of blood cholesterol have high incidence of atherosclerosis, a chronic disease in which cholesterol, cholestryl ester and cellular debris accumulate on the inner surface of the arteries.
- As the disease progresses the deposits reduce or even stop the flow of blood causing coronary artery disease. If this interruption of blood flow occurs in arteries of the heart, the cells irrigated by the affected artery are deprived of oxygen and nutrients and rapidly die. This condition is referred to as heart attack.
- Note that heart problems related to fat metabolism are not generally encountered in animals, which may be due to the following reasons.

Species variation

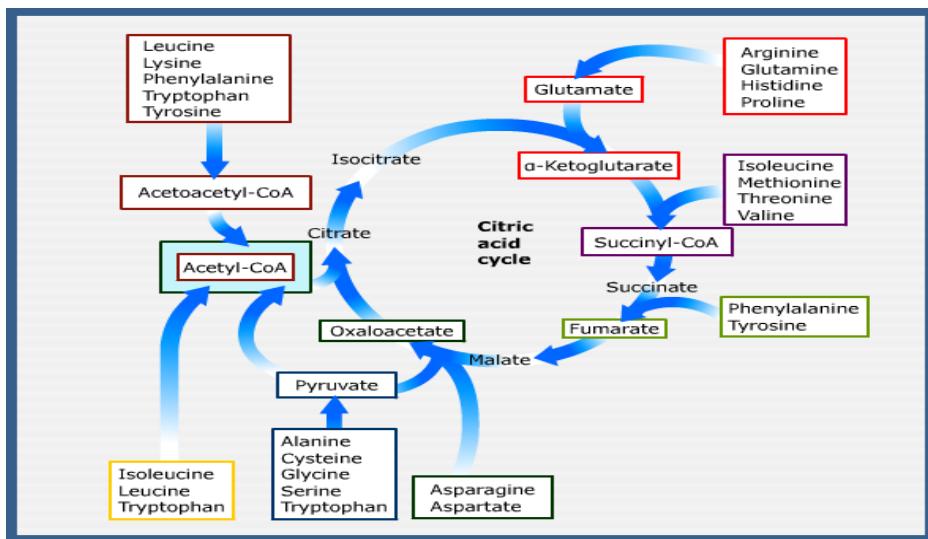
- There are species variation in lipoprotein profiles and the percentage of total cholesterol carried by each lipoprotein type.
- In human the majority of the cholesterol is transported as LDL.
- In pigs, more than 50% of the cholesterol is transported as LDL and VLDL.
- Cattle have approximately equal concentration of HDL and LDL.
- In sheep and horse the majority of cholesterol is circulated as HDL.
- Cats and dogs have highest level of HDL (5-6 times greater than LDL). Dogs and cats are generally resistant to atherosclerosis. This is partly due to the very low concentration of LDL. Hence, serum lipoprotein analyses are not done generally in animals.

AMINO ACID AND PROTEIN METABOLISM

- The protein digestion begins in the stomach and is completed in the intestine. Proteolytic enzymes like proteases and peptidases degrade dietary proteins into their corresponding amino acids.
- The amino acids are then absorbed from the intestinal lumen through secondary active Na^+ dependent transport, through facilitated diffusion and through transport linked to the gamma -glutamyl cycle.
- Major functions of carbohydrates and triacylglycerols are to provide energy, but the primary role of amino acid is to serve as building blocks in the synthesis of tissue proteins. Protein is used secondarily as fuel.
- A diet rich in protein and amino acids, when ingested in excess of the body's requirements, the surplus may be catabolized, since, amino acids cannot be stored.
- Amino acid degradative pathways are similar in most of the organisms.
- When the amino acids are degraded, the carbon skeletons of the amino acids enter the citric acid cycle and from there, they are either oxidized to produce energy or channeled into gluconeogenesis.
- Animals convert nitrogen from amino acids and other sources to one of the three end products namely, ammonia, uric acid or urea, which depends upon the availability of water.
- Most aquatic species such as the bony fishes excrete amino nitrogen as ammonia and are thus called "*ammonotelic*".
- Most land animals excrete amino nitrogen in the form of urea and are hence "*ureotelic*".
- Birds and reptiles excrete amino nitrogen as uric acid and are called "*uricotelic*".

NITROGEN BALANCE

- It refers to the difference between total nitrogen intake and total nitrogen loss.
- Nitrogen equilibrium occurs, when the amount of nitrogen consumed equals to that of excreted mainly in urine, sweat and feces. E.g. Healthy adults are in nitrogen equilibrium.
- A *positive nitrogen balance* occurs when the amount excreted is less than the amount consumed. E.g. growing infants and pregnancy.
- A *negative nitrogen balance* occurs when the amount of nitrogen excreted is more than the amount consumed. E.g. It is associated with inadequate dietary proteins, lack of essential amino acids, cancer, burns and post- surgical patients.



AMINO ACID METABOLISM

- Amino acids obtained from dietary proteins or from the degradation of intracellular proteins are continually degraded.
- There are 20 amino acids in proteins. Correspondingly there are 20 different catabolic pathways for amino acid degradation.
- The first step in amino acid degradation is the removal of α - amino group and the resulting carbon skeleton is metabolized in the citric acid cycle.
- The 20 catabolic pathways produce one of the six metabolic intermediates namely pyruvate, α -ketoglutarate, succinyl-CoA, fumarate, oxaloacetate or acetyl-CoA, all of which enter the citric acid cycle. From here the carbons can be diverted to gluconeogenesis or ketogenesis or they can be completely oxidized to CO_2 and H_2O .
- The division between glucogenic and ketogenic is not very strict. Some amino acids are listed more than once because degradation of these amino acids yields different products, which enters the citric acid cycle at different point.
- There are three categories of amino acid catabolism.
 - Amino acids are designated as glucogenic if they can be converted to glucose
 - Ketogenic, if they can be converted to ketone bodies (only e.g. is Leucine) and
 - Both glucogenic and ketogenic if they can be converted to both types of compounds.
- **Glucogenic:** Amino acids that produce pyruvate or one of the intermediates of citric acid cycle are called glucogenic.
- **Ketogenic:** Those that form acetyl-CoA or acetoacetate can contribute to the formation of fatty acid or ketone bodies.
- The following amino acids are broken down to pyruvate: glycine, alanine, cysteine, serine, threonine and tryptophan.

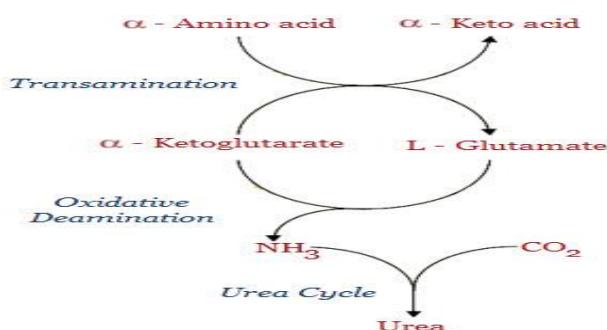
- The following amino acids form acetyl-Co A / acetoacetyl-Co A: The acetoacetyl-CoA is then converted to acetyl-Co A: Phenylalanine, tyrosine, tryptophan, lysine and leucine.
- Four amino acids are converted to succinyl-Co A: methionine, isoleucine, threonine and valine
- The following amino acids are converted to α -ketoglutarate: arginine, histidine, glutamate, glutamine and proline.
- Asparagine and aspartate are degraded to oxaloacetate.
- A part of tyrosine and phenylalanine are converted to fumarate.

Decarboxylation reaction of Amino acids

- Decarboxylation is a reaction in which carboxyl group is removed as CO_2 .
- The enzymes that catalyze the reactions are generally called as decarboxylases. Pyridoxal phosphate is required as coenzyme for all decarboxylases except Histidine decarboxylase.
- Following are some of the decarboxylation reactions, resulting in the production of useful substances.
 - Tyrosine \rightarrow Catecholamine (hormones)
 - Glutamate \rightarrow γ -amino butyrate (Inhibitory neurotransmitter)
 - Tryptophan \rightarrow Serotonin (neurotransmitter /vasodilator)
 - Histidine \rightarrow Histamine (Vasodialator)
 - Methionine \rightarrow Spermine (Involved in DNA packaging)
 - Ornithine \rightarrow Spermidine (Involved in DNA packaging)

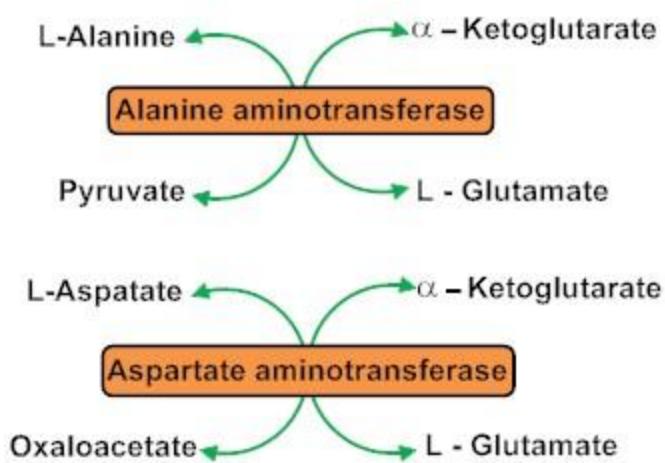
FATE OF AMINO NITROGEN

- Free amino acids derived from dietary proteins or from the body's own proteins, are metabolized in identical ways. Their α -amino nitrogen is first removed either by transamination or by oxidative deamination.
- Alanine and glutamine accounts for over 50% of the total α -amino nitrogen released from muscle tissue. Liver is the primary site for uptake of alanine and the gut for glutamine.



TRANSAMINATION

- Transamination is the major process for the removal of nitrogen from amino acid, which is a reversible reaction.
- α -Ketoglutarate plays an important role in amino acid metabolism by accepting the amino groups from other amino acids forming glutamate.
- The first step in the catabolism of most amino acids is the transfer of their α -amino group to α -ketoglutarate. The products are an α -keto acid (derived from the original amino acid) and glutamate.
- Glutamate produced by transamination can be oxidatively deaminated or can be used as an amino group donor in the synthesis of non-essential amino acids.
- Hence, like TCA cycle, transamination reaction is also amphibolic in nature.
- A family of enzymes called transaminases or aminotransferases catalyzes this transfer of amino groups from one carbon skeleton to another.
- All aminotransferases require coenzyme, pyridoxal phosphate (a derivative of vitamin B₆) for their activity.
- All amino acids with the exception of lysine and threonine participate in transamination reaction.
- Each aminotransferase is specific for one or at the most, a few amino group donors.
- Aminotransferases are named based on the specific amino group donor, because the acceptor is always α -ketoglutarate.
- Two most important transamination reactions are catalyzed by
 - Alanine aminotransferase (ALT) and
 - Aspartate aminotransferase (AST).



Alanine amino transferase

- It is also called as glutamate pyruvate transaminase (GPT).
- It 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 reversible. Thus, glutamate acts as a collector of nitrogen from alanine.

Aspartate amino transferase

- It is also called as glutamate oxaloacetate transaminase (GOT).
- During amino acid catabolism, aspartate aminotransferase transfers amino group from glutamate to oxaloacetate, forming aspartate, which itself used as a source of nitrogen in the urea cycle.
- In most of the reactions α -ketoglutarate receives amino group from other amino acids.
- Here, amino group is transferred from glutamate.

Diagnostic value of Aminotransferases

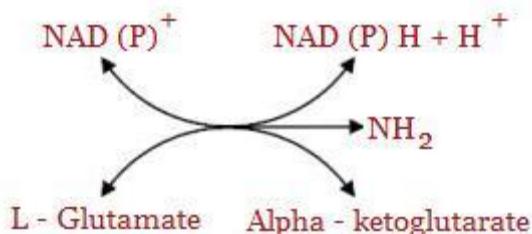
- Aminotransferases are normally intracellular enzymes.
- The elevated levels of aminotransferases in the plasma indicate damage to cells rich in these enzymes.
- For example, physical trauma or a disease process can cause cell lysis, resulting in the release of intracellular enzymes into the blood.
- Plasma AST and ALT are elevated in nearly all - liver diseases and also in non - hepatic disease, such as myocardial infarction and muscle disorders.

OXIDATIVE DEAMINATION

- In contrast to transamination reactions, oxidative deamination results in the liberation of the amino group as free ammonia.
- These reactions occur primarily in the liver and kidney and provide α -keto acid (which can enter the central pathway of energy metabolism) and ammonia (which is a source of nitrogen in urea cycle).
- Glutamate is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by glutamate dehydrogenase, which uses either NAD⁺ or NADP⁺ as coenzyme. The reaction is reversible and functions in both amino acid catabolism and biosynthesis. The reaction occurs only in the mitochondrial matrix.
- When energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, providing substrate for the TCA cycle.
- The sequential action of transamination (resulting in the transfer of amino groups from other amino acids into α -ketoglutarate to produce glutamate) and the subsequent oxidative

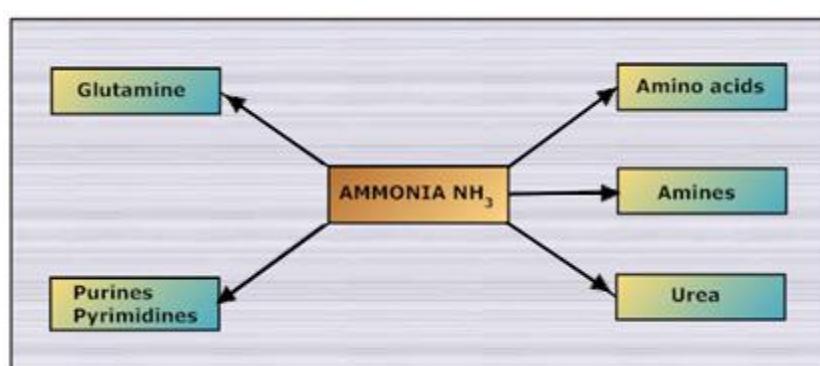
deamination of glutamate (regenerating α -ketoglutarate) result in the release of amino groups of most amino acids as ammonia.

- The combined action of the aminotransferase and glutamate dehydrogenase is referred to as transdeamination.



AMMONIA PRODUCTION AND TRANSPORT

- Ammonia (NH₃) is produced from the amino acid by the reaction catalyzed by glutamate dehydrogenase.
- In kidney, the ammonia is produced from glutamine by renal glutaminase.
- In ruminants, ammonia is also formed by the bacterial degradation of urea.
- Ammonia is highly toxic, particularly affecting the central nervous system. Hence, the free ammonia is converted into 3 non-toxic metabolites, by the following routes and gets transported in blood in any of these forms:
 - Synthesis of glutamate catalyzed by glutamate dehydrogenase.
 - Synthesis of glutamine, ammonia is enzymatically combined with glutamate to yield glutamine in a reaction catalyzed by glutamine synthetase.
 - Synthesis of urea in urea cycle.



- In brain, mostly NH₃ combines with glutamate to form glutamine, which is the major transport form of ammonia.
- Most of the NH₃ generated in catabolism is present as NH₄⁺ at neutral pH. It is a weak acid and ammonia is strong base.
- Since the toxic ammonia is converted to urea in urea cycle, any defects in urea cycle will result in ammonia toxicity.

UREA CYCLE

- Hans Krebs and Kurt Henseleit elucidated this pathway in 1932.
- Hence it is also called as “Krebs – Henseleit cycle”.
- This is also known as “Ornithine cycle” or “Ornithine – Urea cycle”.
- In urea cycle two molecules of ammonia and one molecule of CO₂ are fixed to form urea.
- In mammals, the synthesis of urea or ureogenesis occurs mainly in the liver.
- Urea formed in the liver is then transported in the blood to the kidneys for excretion in the urine.
- Urea is the product of a set of five enzymatic reactions.
- The first two reactions occur in the mitochondria whereas the remaining reaction takes place in the cytoplasm.
- Fumarate released in the urea cycle links urea cycle with TCA cycle.

Reactions of Urea Cycle occur in mitochondria and cytosol

Reactions in mitochondria

- **1st reaction:** The ammonia released by oxidative deamination of glutamate in mitochondria reacts with bicarbonate to form carbamoyl phosphate.
- This reaction requires two molecules of ATP and is catalyzed by carbamoyl phosphate synthetase I.
- N-acetyl glutamate acts as a positive allosteric effector.
- **2nd reaction:** Carbamoyl phosphate and Ornithine are condensed to form Citrulline in a reaction catalyzed by Ornithine transcarbamoylase.
- Citrulline is then transported to cytosol.

Reactions in cytosol

- **3rd reaction:** Citrulline condenses with aspartate to form arginosuccinate, in the presence of ATP, which is converted to AMP and PPi. This reaction is catalyzed by arginosuccinate synthetase (the second nitrogen atom for urea synthesis comes from aspartate).
- **4th reaction:** Arginosuccinate is cleaved to form arginine and fumarate catalyzed by arginosuccinate lyase.
- Arginine is the immediate precursor of urea.
- Fumarate is hydrated to malate, which is transported into the mitochondria and enters the TCA cycle.
- **5th reaction:** In this reaction arginine is hydrolytically cleaved to form ornithine and urea, catalyzed by arginase.

- Ornithine is transported into the mitochondria, where it reacts with carbamoyl phosphate to continue the operation of urea cycle.

Overall reaction

- Aspartate + $\text{NH}_3 + \text{CO}_2 + 3\text{ATP} \rightarrow \text{Urea} + \text{Fumarate} + 2\text{ADP} + \text{AMP} + 2\text{Pi} + \text{PPi} + 3\text{H}_2\text{O}$.
- Four high-energy phosphate bonds are consumed (equivalent to 4 ATP) in the synthesis of each molecule of urea.

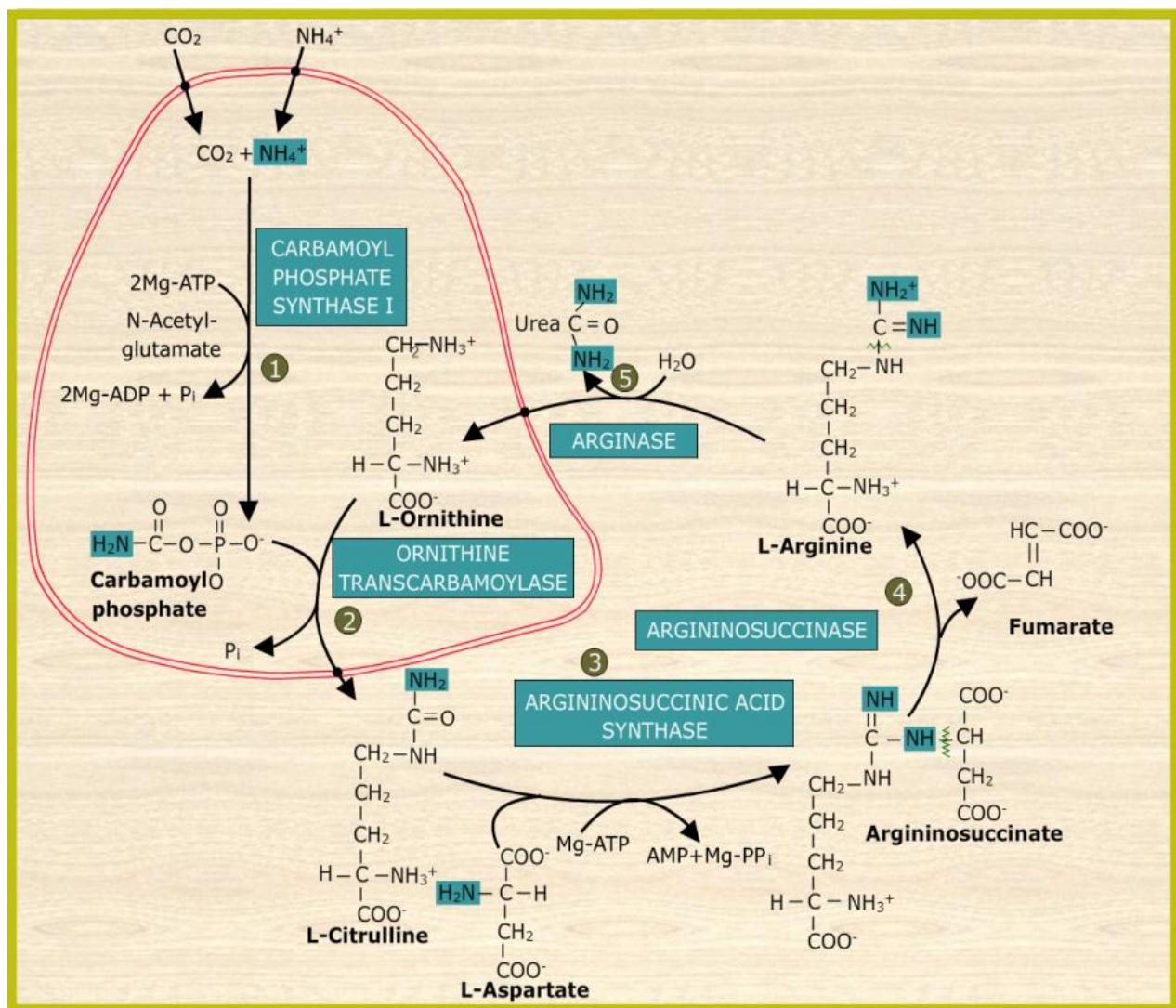


Fig: REACTIONS OF UREA CYCLE

Clinical Significance

- Some genetic defects in urea synthesis (due to lack of enzymes) will lead to an elevated level of NH_4^+ . The affected individual becomes lethargic, has vomition, and there is irreversible brain damage. In liver failure, liver is unable to take up NH_4^+ from the blood causing an elevated ammonia concentration in blood.

- In brain tissue, NH_4^+ combines with α -ketoglutarate (a component of citric acid cycle) to form glutamate, catalyzed by glutamate dehydrogenase and glutamate is converted to glutamine, by glutamine synthetase. Deficiency of α -ketoglutarate causes a reduction in cellular oxidation and ATP formation, causing brain damage. Over production of glutamate and glutamine may also cause brain damage.

PROTEIN DEGRADATION

- Proteins undergo continuous turn over (synthesis and degradation.). Degradation prevents the accumulation of abnormal and unwanted proteins.
- Proteins may have specific sequences that are recognized by proteolytic machinery for the degradation.
- Proteins have different half-lives. Some proteins have longer half - lives (days). (E.g. Hb) and some proteins may have shorter half-lives (hours or minutes). (E.g. Defective proteins, damaged proteins and the enzymes that regulate the metabolic pathways).
- The half-life of a protein is generally determined by its amino terminal residue. Proteins with any one of the following amino acids, methionine, glycine, alanine, serine, threonine and valine, as an N-terminal residue have the half-life of more than 20 hours, whereas proteins with arginine, leucine, phenylalanine, aspartic acid and lysine, as N- terminal amino acid will have half life of about 2 -3 minutes.
- Proteins rich in amino acids - proline, glutamate, serine and threonine (called PEST sequence considering the one letter designation of these amino acids) are rapidly degraded. These proteins have short half-lives.
- Proteins are degraded by two major pathways:
- 1. Energy dependant ubiquitin proteosome pathway (UPP), which degrades proteins synthesized within the cells (intracellular proteins).
- 2. Non-energy dependant lysosomal pathway, which degrade extracellular proteins and some cell surface proteins.
- Ubiquitin is a small protein present in all eukaryotic cells. The carboxy terminal glycine of ubiquitin is attached to ϵ - amino group of lysine of protein to be degraded (Ubiquitination).
- ATP supplies energy for this process.
- Ubiquitination targets proteins to proteosomes for degradation.
- Proteosomes are multisubunit structures, having half the size of ribosomes. It contains sites for proteolytic activity. The targeted proteins are cut into several small fragments, which are further degraded to amino acids.
- Lysosomes are subcellular organelles containing hydrolytic enzymes having maximal hydrolytic activity at pH 4 to 5. The low pH of the lysosomes denature proteins, which increases the susceptibility for degradation.
- The extracellular proteins are taken into the cell by endocytosis and are delivered to lysosomes, wherein they are degraded by hydrolases of lysosomes.

PROTEIN METABOLISM IN RUMINANTS

- Ruminant utilize non-protein nitrogen compounds such as ammonia, urea and biuret, amines and amino acids as a source of nitrogen for amino acid synthesis.
- The microbes present in rumen are capable of synthesizing all essential and non-essential amino acids from non-protein nitrogen.
- Ruminants depend largely on microbial protein to meet their own protein requirements.

The sources of amino acids are

- Those proteins that escape hydrolysis by rumen microbes.
- Those proteins that are constituents of microbes that reach the abomasums (true stomach).
- Fermentation that occurs in the rumen and reticulum of ruminants is carried out by the action of bacteria and protozoa. Bacteria accounts for about 80% of the rumen metabolism. Protozoa accounts for about 20%. The microorganisms are anaerobic.
 - The microorganisms of rumen hydrolyze protein to peptides and finally to amino acids. The amino acids are degraded by the fermentative deamination with the production of CO₂, NH₃ and Short chain / Volatile Fatty Acids. The rumen bacteria synthesize amino acids using ammonia as the principal source of nitrogen.
 - Urea is the waste product of protein catabolism.
 - In ruminants, hepatic urea production is from two sources:
 - Deamination of endogenous amino acid.
 - Nitrogen absorbed as ammonia from rumen.
- Ammonia is delivered to liver where it is converted to urea.
- In monogastric animals, urea is excreted exclusively by the kidney. In ruminants urea is also excreted into rumen. Excretion is directly from the blood or through saliva. In rumen the urea is again converted to ammonia by Urease present in the rumen bacteria, which is known as "Urea Recycling".

GENETIC CODE

- The genetic code is a collection of codons that specify all amino acids found in protein.
- A codon is a sequence of three consecutive bases (triplet) in mRNA (5' to 3') that specify a particular amino acid during translation.
- The successive codons in mRNA determine the sequence in which amino acids are added to the growing polypeptide chain.
- Similarly, the tRNA has triplet complementary bases (anticodons, 5' to 3') that can read these codons and transfer the corresponding amino acid to ribosomes during protein synthesis.

Salient features of Genetic Code:

- The genetic code was deciphered by Hargobind Khorana.
- **The genetic code is degenerate** (redundant). There are 64 codons in the genetic code out of which 61 codons specify an amino acid and the remaining 3 act as stop codons for protein synthesis. There is at least one codon for each amino acid. Many amino acids have more than one codon i.e. degeneracy of the codon. Codons that specify the same amino acid are called **synonyms**. For example CAU and CAC are synonyms for Histidine.
- One triplet base codes for only one amino acid and so the code is **unambiguous and specific**.
- When two or more codons specify the same amino acid, they differ in the third base (wobble) of the triplet.
- The genetic code is **non-overlapping** (each nucleotide is used only once).
- The code is **comma less** (there are no marks to distinguish one codon from the next).
- The code is nearly **universal**. The same codon specifies the same amino acid in almost all species. However some differences have been found in the codons in mitochondria.

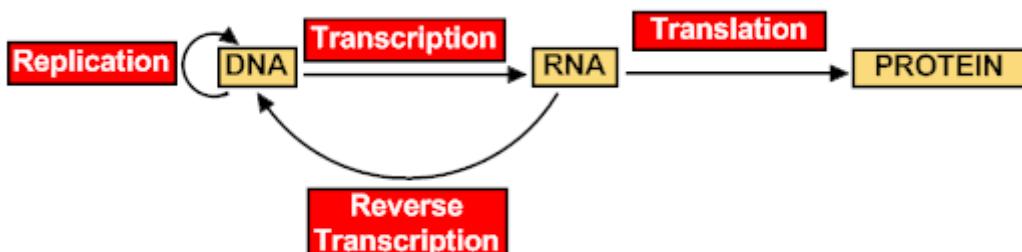
Start and Stop Codons:

- The start signal for protein synthesis is AUG (called as 'Start codon' or 'Initiation codon'), which specifies the incorporation of methionine at 5' end of mRNA in eukaryotes and N-f-Methionine (N-formyl-met) in prokaryotes and mitochondrial protein synthesis.
- The three codons UAA (amber), UAG (ochre) and UGA (opal) are called as 'Stop' or 'Terminating' or 'Non-sense' codons for protein synthesis, which are seen at 3' region of mRNA.

The genetic code along with respective amino acids						
First base 5' end	Second base (middle one)				Third base 3' end	
	U	C	A	G		
U	UUU Phe UUC UUA Leu UUG	UCU Ser UCC UCA UCG	UAU Try UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	
	CUU CUC CUA Leu CUG	CCU Pro CCC CCA CCG	CAU His CAC CAA Gln CAG	CGU Arg CGC CGA CGG	U C A G	
	AUU Ile AUC AUA AUG Met	ACU Thr ACC ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G	
	GUU Val GUC GUA GUG	GCU Ala GCC GCA GCG	GAU Asp GAC GAA Glu GAG	GGU Gly GGC GGA GGG	U C A G	

Central dogma:

- This was originally proposed by Crick which states that the information contained in the base sequence of DNA is transferred to RNA and then to protein. The information is transferred in one direction.



- An exception to this rule is found in retrovirus, which has an enzyme called reverse transcriptase which can copy RNA into DNA.

WOBBLE HYPOTHESIS

- This explains how tRNA can recognize more than one codon for a specific amino acid.
- There are 61 codons that specify the 20 different amino acids. But, there are not 61 tRNAs (about 40 tRNAs in prokaryotes & 50 tRNAs in H.beings).
- During protein synthesis each codon is recognized by a triplet base called anticodon of tRNA.
- The wobble hypothesis suggests that the first two bases in the codon of mRNA base pairs with its complementary bases of the anticodon in tRNA.
- The last base of the codon pairs loosely with its corresponding base in the anticodon.
- The first base of anticodon (5' end) is paired with the third base of the codon (3' end).

- The 5' position of the anticodon of some tRNA contains the modified base inosine (I). 'I' can base pair with any of U, C, or A in the third position of codon. (Note that mRNA codon is read in 5'→3' by an anticodon pairing 3'→5' direction. (When writing the sequences of both the codons and anticodons the sequences must be written in the 5'→3' direction only).
- When an amino acid is coded by more than one codon, for example alanine (GCU, GCC and GCA) can pair with a single tRNA that contain the anticodon (5'—IGC—3'). Hence, minimum of 32 tRNAs are required to translate all 61 codons.
- Two generalization concerning the codon-anticodon interaction can be made:
 - The first two bases of codon pair in the standard way. Codon that differs in either of their first two bases must be recognized by different tRNAs.
 - The first base of an anticodon determines whether a particular tRNA molecule reads one, two or three kinds of codon. C or A (1 codon), U or G (2 codons) or I (3 codons).

PROTEIN BIOSYNTHESIS (TRANSLATION)

Protein synthesis in prokaryotes

- Translation is a process by which cells synthesize proteins. It is a complex process.
- During the synthesis of proteins the mRNA is read in the 5' to 3' direction and the protein is synthesized in the N-terminal to C-terminal direction.
- All the three types of RNAs are needed for translation.
- The mRNA contains codons, which determines the sequence of amino acid in a protein (i.e. acts as template for protein synthesis).
- Transfer RNAs (tRNA) are adapter molecules that decode the codons in mRNA and carry amino acids to the site of protein synthesis - ribosomes.
- Ribosomal RNA (rRNA) form part of the ribosome that brings together all the components necessary for protein synthesis.
- Several enzymes are also involved in protein synthesis.

Translation can be divided in to three stages:

- Activation of amino acids.
- Protein synthesis proper (Initiation, Elongation & Termination).
- Post-translational modifications.

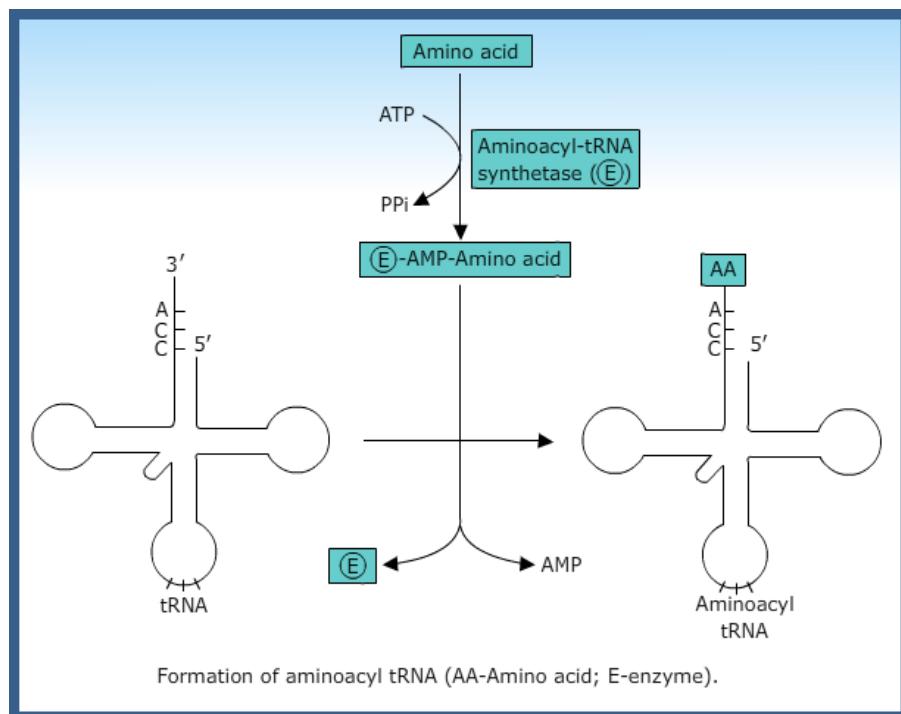
COMPONENTS REQUIRED FOR PROTEIN SYNTHESIS

- **Amino acids** (all the 20 amino acids),
- **Ribosomes** : Ribosome is the cell's factory for protein synthesis.
 - Each ribosome consists of two subunits. One is larger (50 S) and the other is smaller (30 S)
 - Ribosome contain enzymes.
 - Ribosome has binding sites for mRNA and tRNA.
 - Each ribosome has three binding sites for tRNA
 - The aminoacyl site (A site)
 - The peptidyl site (P site)
 - The exit site (E site)
 - An incoming tRNA with its linked amino acid occupies the aminoacyl site (A-site).
 - And the tRNA attached to the growing polypeptide chain occupies the (P-site) peptidyl site.
 - The deacylated tRNA occupies the "E site".
- **mRNA,**
- **tRNA,**
- **Energy sources** (ATP, GTP) and
- **Accessory protein factors.**

ACTIVATION OF AMINO ACIDS

Attachment of an amino acid to its tRNA

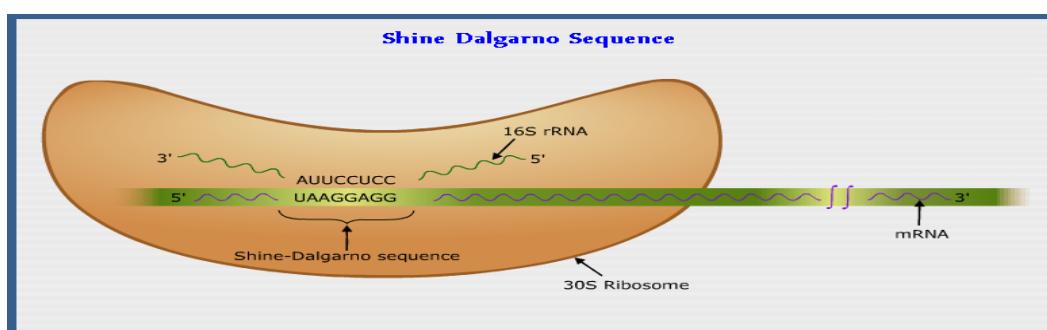
- Amino acids are not directly incorporated into proteins on the mRNA template. Amino acids are activated and attached to their corresponding tRNA by an enzyme known as aminoacyl - tRNA synthetase.
- This is a two-step reaction.
- Each aminoacyl-tRNA synthetase recognizes a particular amino acid and binds with the amino acid. This process is carried out by the hydrolysis of ATP.
 - Amino acid + ATP + Aminoacyl tRNA synthetase (E) → E - AMP- Amino acid
 - tRNA + E - AMP- Amino acid → AMP + E + aminoacyl tRNA ("charged" tRNA)
- All the tRNA molecules have at their 3' end the nucleotide sequence CCA. An amino acid is joined to its tRNA through an ester bond between its carboxyl group and either 2' or 3' hydroxyl group of the ribose of the terminal adenosine moiety.



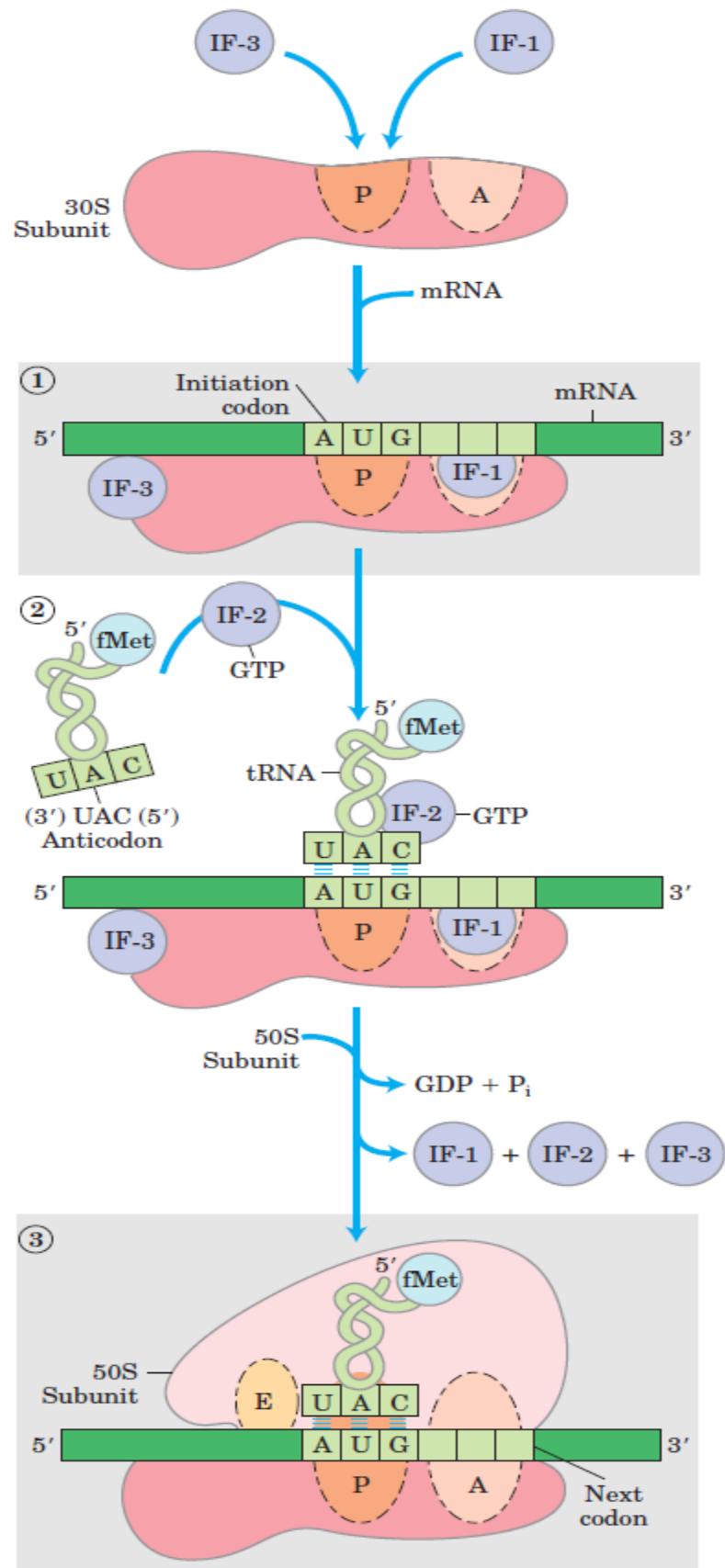
INITIATION OF PROTEIN SYNTHESIS

Initiation

- In this process the components involved in protein synthesis are assembled.
- The first step involves the binding of the small ribosomal subunit to the mRNA at a specific point upstream of AUG.
- In prokaryotes, a purine rich sequence of nucleotide bases (5'-UAGGAGG3') known as '**Shine – Dalgarno**' sequence is located 6 to 10 bases upstream of the initiator AUG codon on the mRNA. These sequences can base pair with the 3' end of the 16S rRNA of the 30S ribosomal subunit (5' end of mRNA and the 3' end of rRNA can form complementary base pairing). This helps the binding and positioning of the mRNA on the 30S ribosomal subunits.



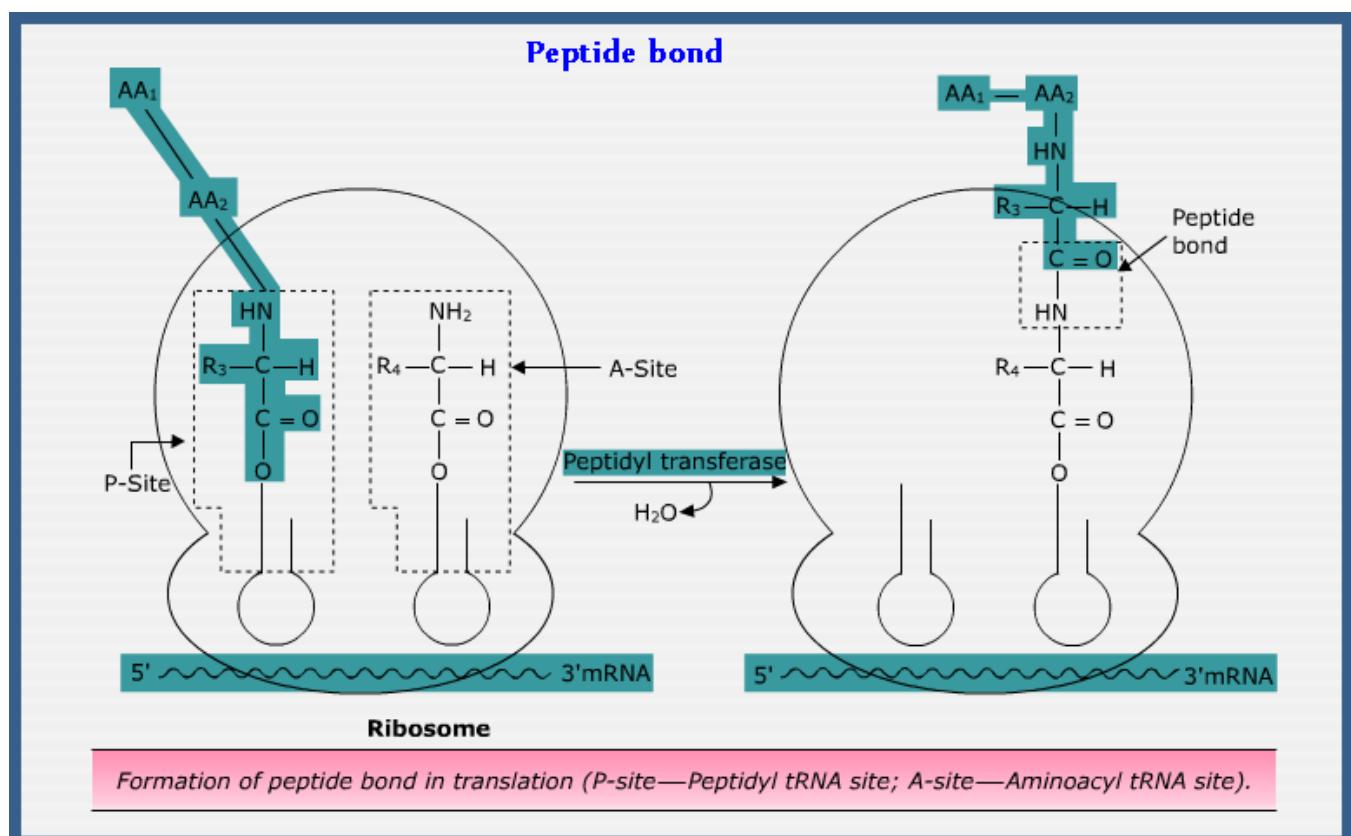
- Initiation factors required are IF1, IF2 and IF3.
- Translation usually begins at the sequence of AUG, which codes for methionine and is known as initiation codon.
- In bacteria, the methionine that initiates protein is formylated (f-met) and is carried by tRNA_{f-met}.
- There are two different tRNAs that recognize AUG and carry methionine. One is used for formylated methionine (tRNA_{fmet}) and the other recognizes methionine - internal AUGs (tRNA_{met}).
- Once the mRNA is positioned in the 30S subunit of ribosome, IF1 attaches to the 'A' site, preventing the attachment of initiating methionyl tRNA_{f-met} at the 'A' site.
- Now IF3 also attaches on the 30S subunit, preventing the premature attachment of 50S subunit. Therefore IF3 is also known as 'Anti-association factor'.
- Next IF2 complexed with GTP attaches to methionyl tRNA_{f-met}.
- The anti codon of the above complex binds with the initiation codon, present at the 'P' site forming a larger complex. This is the only aminoacyl tRNA that can attach to the 'P' site. All others will attach to 'A' site only.
- Now the 50S subunit attaches to the 30S complex, which requires energy supplied by the hydrolysis of GTP, with simultaneous release of IF1, IF2, IF3, GDP and Pi.
- Thus the initiation complex is formed.
- Initiation complex contains - 70S ribosome, mRNA and methionyl tRNA_{f-met}.



ELONGATION OF PROTEIN SYNTHESIS

Elongation

- The elongation factors required are EF-Tu, EF-Ts and EF-G.
- This process involves the addition of amino acids to the carboxyl end of the growing polypeptide chain.
- The mRNA codon at the 'A' site determines which amino acyl- tRNA should enter (this is achieved by codon-anticodon recognition –i.e. mRNA-tRNA complementary base-pair reading).
- Next the aminoacyl tRNA binds with EF-Tu -GTP complex, which in turn basepair with the codons, in the 'A' site. The process requires energy, which is supplied by the hydrolysis of GTP, releasing GDP, Pi and EF-Tu.
- The exact role of EF-Ts is not clear; but it is assumed that EF-Ts replenish the EF-Tu-GTP complex.
- The tRNA carrying the corresponding specific amino acid enters at the 'A' site and base pairs with the second codon.



- Now both 'A' and 'P' sites are occupied. The enzyme peptidyltransferase catalyzes the formation of peptide bond between the carboxyl group of methionine and the amino group of second amino acid, leaving the amino group of initiating methionine free.
- Thus the 'P' site will be occupied by deacylated tRNA and 'A' site will be occupied by dipeptidyl tRNA.
- Now, the ribosome moves one codon forward, shifting the deacylated tRNA to 'E' site, dipeptidyl tRNA to 'P' site and the 'A' site with next codon will be free to accept the new aminoacyl tRNA.
- This movement of ribosome (translocation) needs a third elongation factor - G (EF-G) and GTP. EF-G is also known as 'Translocase'. GTP is hydrolysed to GDP + Pi.
- This process continues, completing the elongation cycle.
- As translation proceeds, the ribosome moves along the mRNA. The start AUG codon is free to bind to a new ribosome. Thus mRNA is covered by several ribosomes. The resultant structure is called as polyribosome or polysome.

TERMINATION OF PROTEIN SYNTHESIS

Termination

- There are three codons UAA, UAG and UGA that have no corresponding tRNA molecules.
- These codons are called 'Stop codons' or 'Non-sense codons'.
- The translation ends when a termination codon enters the 'A' site.
- Now the 'Releasing factors' (RF), attach and release all the components of the protein synthesising complex, the newly synthesised polypeptide chain, the ribosomal subunits, mRNA and tRNA.
- Instead of tRNA interacting on 'A' site, the site is now filled with Releasing factors. In E.coli RF1 and RF2 perform this function, which is assisted by RF3.
- Generally, RF-1 recognizes the stop codons - UAG and UAA; RF-2 recognizes UGA and UAA; the specific function of RF-3 is not known, but is believed to release the ribosomal subunits.

FACTORS REQUIRED FOR TRANSLATION

Stage of protein synthesis	Prokaryote	Eukaryote
Initiation factors	IF-1, IF-2 & IF-3	eIF-1 through eIF-9
Elongation factors	EF-Tu, EF-Ts & EF-G	eEF-1 α , eEF-1 β & eEF-2
Termination factors	RF-1, RF-2 & RF-3	eRF

POST - TRANSLATIONAL MODIFICATIONS

- After the synthesis of proteins, some chemical modifications occur on the structure of proteins to generate a functional protein. These changes are collectively known as post-translational modifications.
- The changes include cleavage of polypeptide and various covalent attachments of chemical groups.
- Cleavage of polypeptide chains is a very common modification. It takes place on the N and C termini by amino and carboxypeptidases as well as on the internal peptide linkages.
- Chemical modifications are many, which are found to take place on the N and C termini as well as on the most of the side chains of amino acids. The modification include:
 - Acetylation
 - Hydroxylation
 - Phosphorylation
 - Methylation
 - Glycosylation
 - Addition of nucleotides.

PROTEIN SYNTHESIS IN EUKARYOTES

Synthesis of Proteins in eukaryotes (Differences from prokaryotes)

- **Ribosomes:** They are larger consisting of 60S large subunit and 40S small subunit that will assemble to form 80S ribosomal particle.
- **Initiator tRNA:** The initiation codon is AUG and methionine is not formylated. The aminoacyl-tRNA is called Met-tRNAs_i.
- **Start signal:** AUG nearest to the 5' end of mRNA is usually selected as start site.
- **Initiation:** Initiation in eukaryotes is similar to that of prokaryotes. The factors are designated as eIF1, eIF2. At least 9 initiation factors are involved.
- **Elongation:** Elongation of a polypeptide chain needs three proteins called elongation factors in eukaryotes - eEF-1 α , eEF-1 β & eEF-2 whose role is similar to EF-Tu, EF-Ts and EF-G respectively.
- **Termination:** In eukaryotes, only one Releasing factor-eRF is involved, which requires GTP.

INHIBITORS OF PROTEIN SYNTHESIS

- **Puromycin** causes premature chain termination by acting as structural analogue of aminoacyl-tRNA in both prokaryotes and eukaryotes.

The following compounds (antibiotics) inhibit protein synthesis on 70S ribosomes of prokaryotes:

- **Streptomycin** binds to 30S subunit of prokaryotes and causes misreading of mRNA thereby preventing the formation of initiation complex.
- **Tetracycline** binds to 30S ribosomal subunit and inhibits the binding of aminoacyl -tRNA to the 'A' site.
- **Chloramphenicol** inhibits the peptidyltransferase activity of the 50S ribosomal subunit of prokaryotes.
- **Erythromycin** binds to the 50S ribosomal subunit of prokaryotes and prevents translocation.

The following compounds inhibit protein synthesis on 80S ribosomes of eukaryotes:

- **Cycloheximide** inhibits peptidyltransferase activity of the 60S ribosomal subunit in eukaryotes.
- **Diphtheria toxin** prevents translocation in eukaryotes by inactivating eEF-2.

PURINE METABOLISM

- Dietary nucleic acids are degraded by pancreatic ribonuclease and deoxyribonuclease to mononucleotides.
- They are then converted to nucleosides by mononucleotidase and finally to free bases by nucleosidases. The phosphate and sugar produced by the digestion of nucleic acids are reused.
- Most of the purine and pyrimidine bases are catabolized and excreted. Purines are converted to uric acid and pyrimidine degradation leads to formation of urea.
- Purine and pyrimidine nucleotides are synthesized by two pathways:
 - **De novo synthesis:**

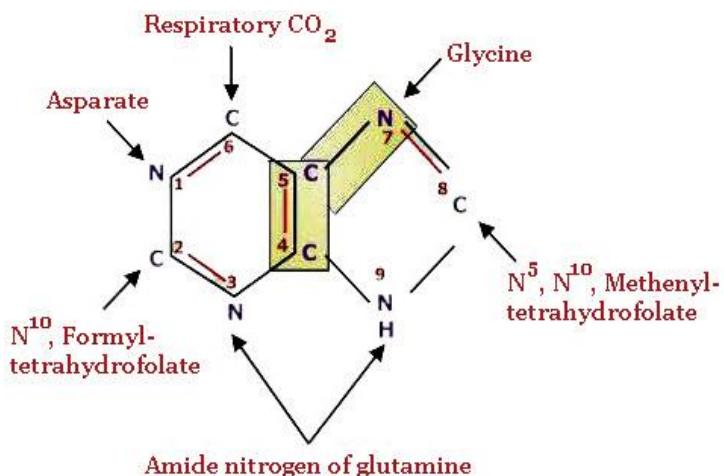
In *de novo* synthesis, purines and pyrimidines are synthesized from the smaller precursor molecules such as glycine, aspartic acid, glutamine, CO₂ and tetrahydrofolic acid. This pathway is expensive; Several reactions require ATP.

- **Salvage pathway:**

In salvage pathway the free bases and nucleosides released during the nucleic acid breakdown are reused.

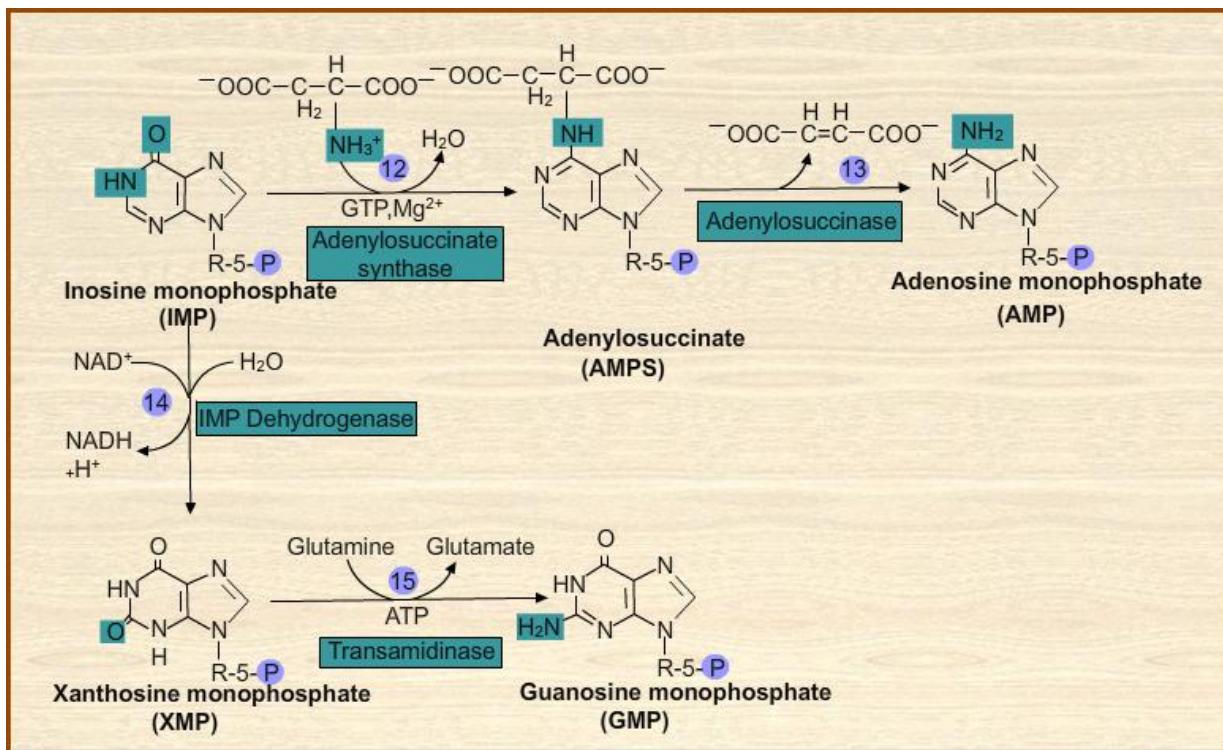
- Both types of pathways are important.

***De Novo* Synthesis of purines**



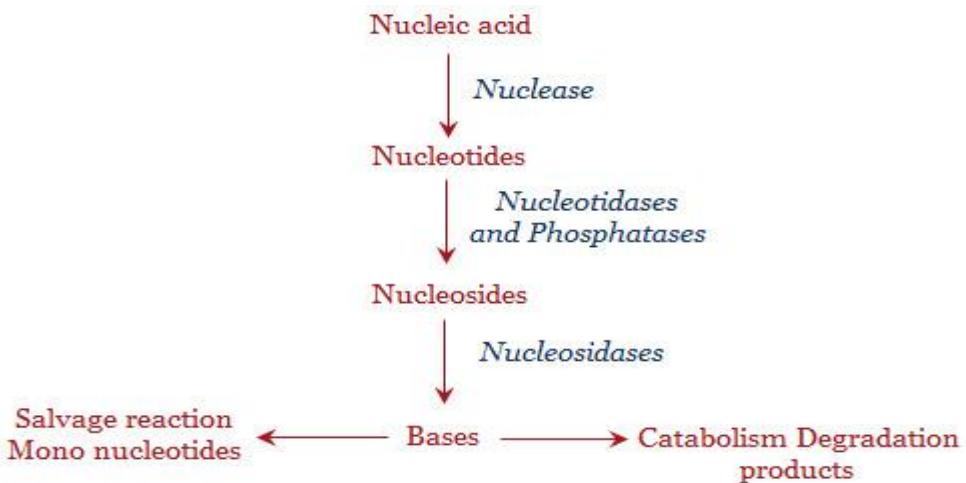
SOURCES FOR PURINE RING

- All enzymes of purine metabolism are found in cytoplasm.
- Purine ring structure is not synthesized as a free base but as a substituent of ribose-5-phosphate, which comes from 5-phosphoribosyl-1-pyrophosphate (PRPP). The PRPP is formed from ribose 5-phosphate and ATP. PRPP then donates the ribose 5-phosphate, which serves as base upon which purine structure is built.
- The first step is the reaction of PRPP with glutamine to form 5-phosphorybosylamine. The C-1 is changed from α to β configuration. This step can be inhibited by azaserine, an antimetabolite of glutamine.
- Glycine is added to ribosylamine, forming glycinate ribosyl 5-phosphate. This reaction requires ATP. The carbons 4,5 and nitrogen 7 are donated by glycine.
- Next, a formyl group is transferred from N^{10} – formyltetrahydrofolate to the amino group of glycine to produce formylglycinamide ribosyl 5-phosphate (Carbon 8).
- The amide group of glutamine is transferred to the aldehyde derivative forming formylglycinamide ribosyl 5-phosphate (Nitrogen 3).
- The next reaction is the ring closure that produces an imidazole ring. This reaction requires ATP.
- CO_2 is incorporated by attachment to the carbon that becomes C-5 of the purine. This reaction requires neither biotin nor ATP. The product is aminoimidazolecarboxylate ribosyl 5 – phosphate.
- The next step is the condensation of amino group of aspartate with the newly added carboxylate to form an amide, aminoimidazole succinyl carboxamide ribosyl 5-phosphate.
- Fumarate is eliminated by a non-hydrolytic cleavage. This results in the transfer of an amino group to become N-1 of IMP.
- N^{10} – formyltetrahydrofolate donates a formyl group to the amino group forming formimidooimidazole carboxamide ribosyl 5-phosphate (Carbon 2).
- Ring closure occurs by the condensation of amide nitrogen with the formyl group to produce IMP.
- The two-purine nucleotides AMP and GMP are subsequently formed from IMP by two different enzymatic reactions.
- The amino group of aspartate condenses with the keto group of IMP to give adenylosuccinate. The energy for this reaction is supplied by GTP.
- The adenylosuccinate is cleaved to produce AMP and fumarate.
- In the conversion of IMP to GMP, C-2 is oxidized to give xanthosine monophosphate in the presence of NAD^+ . Glutamine donates its amide nitrogen to C-2 in an ATP dependant reaction yielding GMP, AMP and PPi.

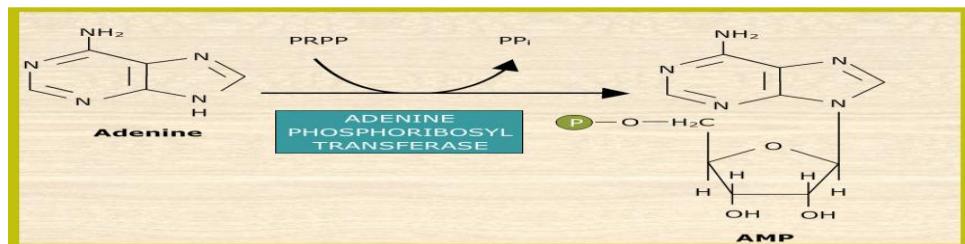


Salvage Pathway

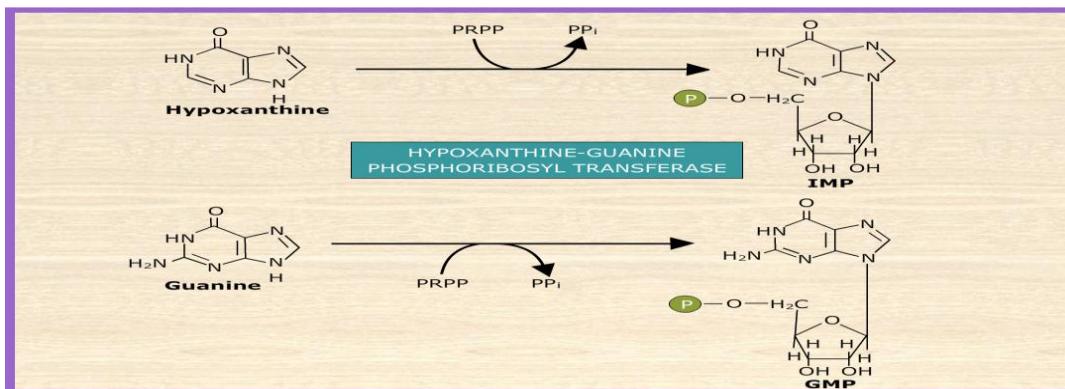
- It is a metabolic pathway that utilizes compounds formed in catabolism for biosynthetic reactions.
- During cellular metabolism and during digestion in animals nucleic acids are degraded to mononucleotides, nucleosides and finally to free bases. Some of the purines and pyrimidines formed in this way are further degraded. But considerable amount is salvaged by reacting with PRPP to reform nucleotides.
- The recycling of bases conserves cellular energy.



- Two specific salvage enzymes participate for the utilization of adenine, hypoxanthine and guanine.
- Adenine phosphoribosyltransferase (APRT) catalyzes the reaction of adenine with PRPP to form AMP and PP_i.



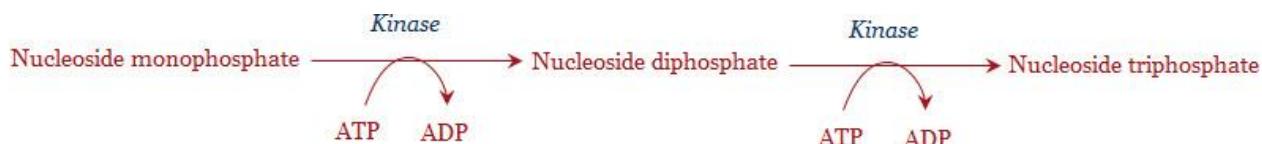
- Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the conversion of hypoxanthine to IMP and guanine to GMP with concomitant formation of pyrophosphates.



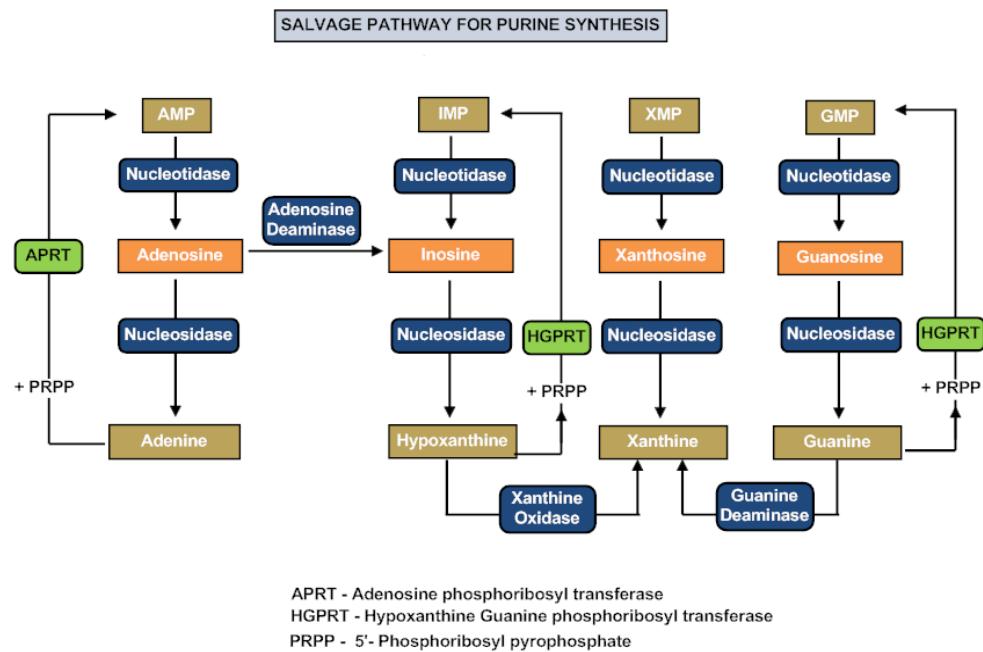
- Lesch-Nyhan syndrome is caused by the deficiency of HGPRT. In this condition, purine bases cannot be salvaged; instead they are degraded, forming excess amounts of uric acid.
- Individual with this syndrome suffer from mental retardation. They are prone to chewing of their fingers and performing other acts of self-mutilation.

NMP to NDP to NTP

- Conversion of nucleotide monophosphate to diphosphate and triphosphate occurs with the help of kinases.

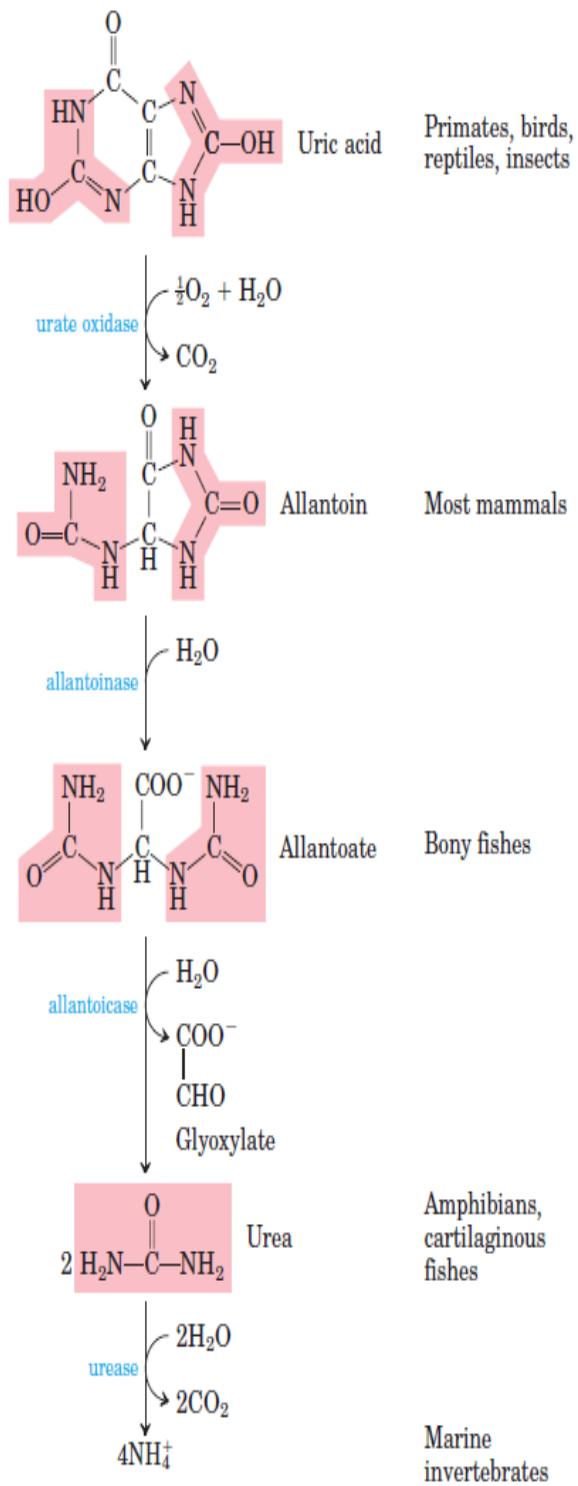
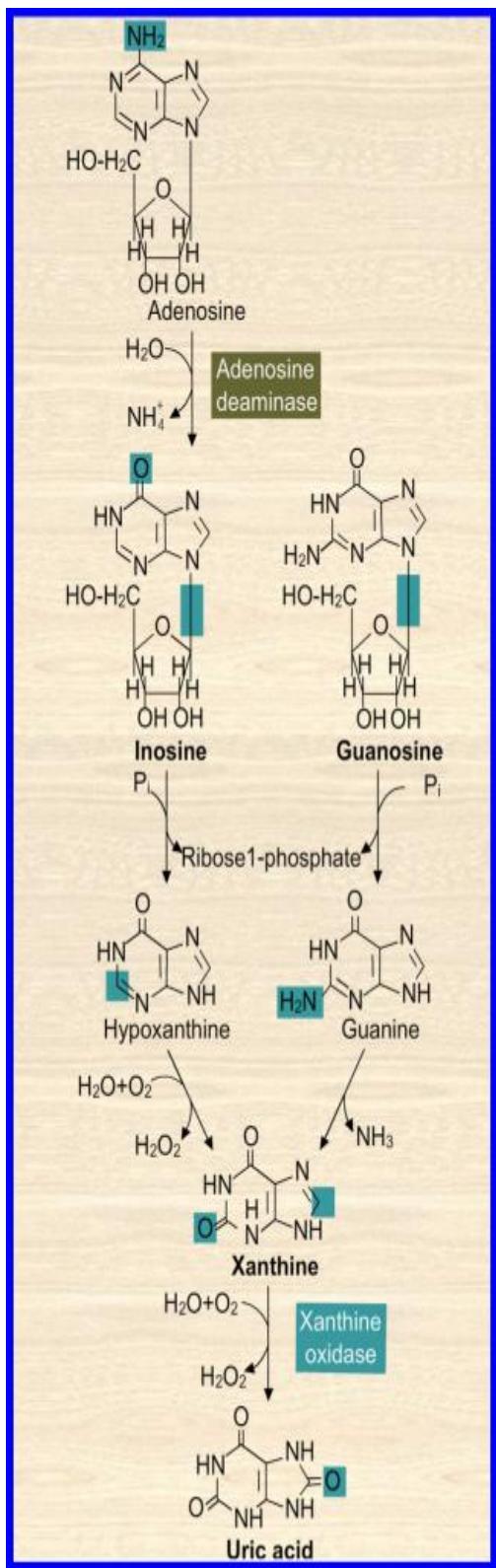


SALVAGE PATHWAY - PURINE SYNTHESIS



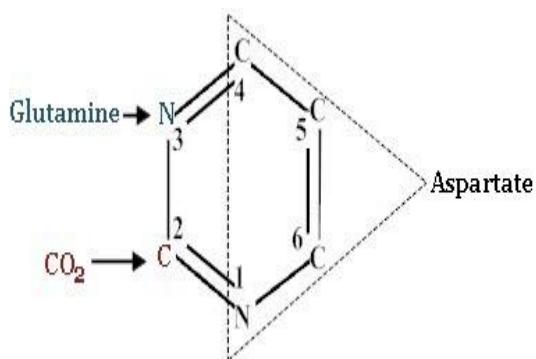
PURINE CATABOLISM

- The first enzyme in AMP degradation is AMP deaminase, which catalyzes the conversion of AMP to IMP and ammonia.
- IMP is hydrolyzed to inosine by the removal of inorganic phosphate followed by the removal of ribose as ribose 1-phosphate catalyzed by the enzyme purine nucleoside phosphorylase to produce hypoxanthine.
- Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine.
- Guanine nucleotide also follows the similar pathway (removal of phosphate and ribose sugar) to produce guanine, which is then deaminated to xanthine catalyzed by guanase or guanine deaminase.
- Finally xanthine is converted to uric acid by the enzyme xanthine oxidase.
- Mammals other than primates and reptiles produce allantoin (highly water soluble) as their end product of purine catabolism. Such organisms contain the enzyme uricase that converts uric acid to allantoin. The following are the other end products of purine catabolism in different species.



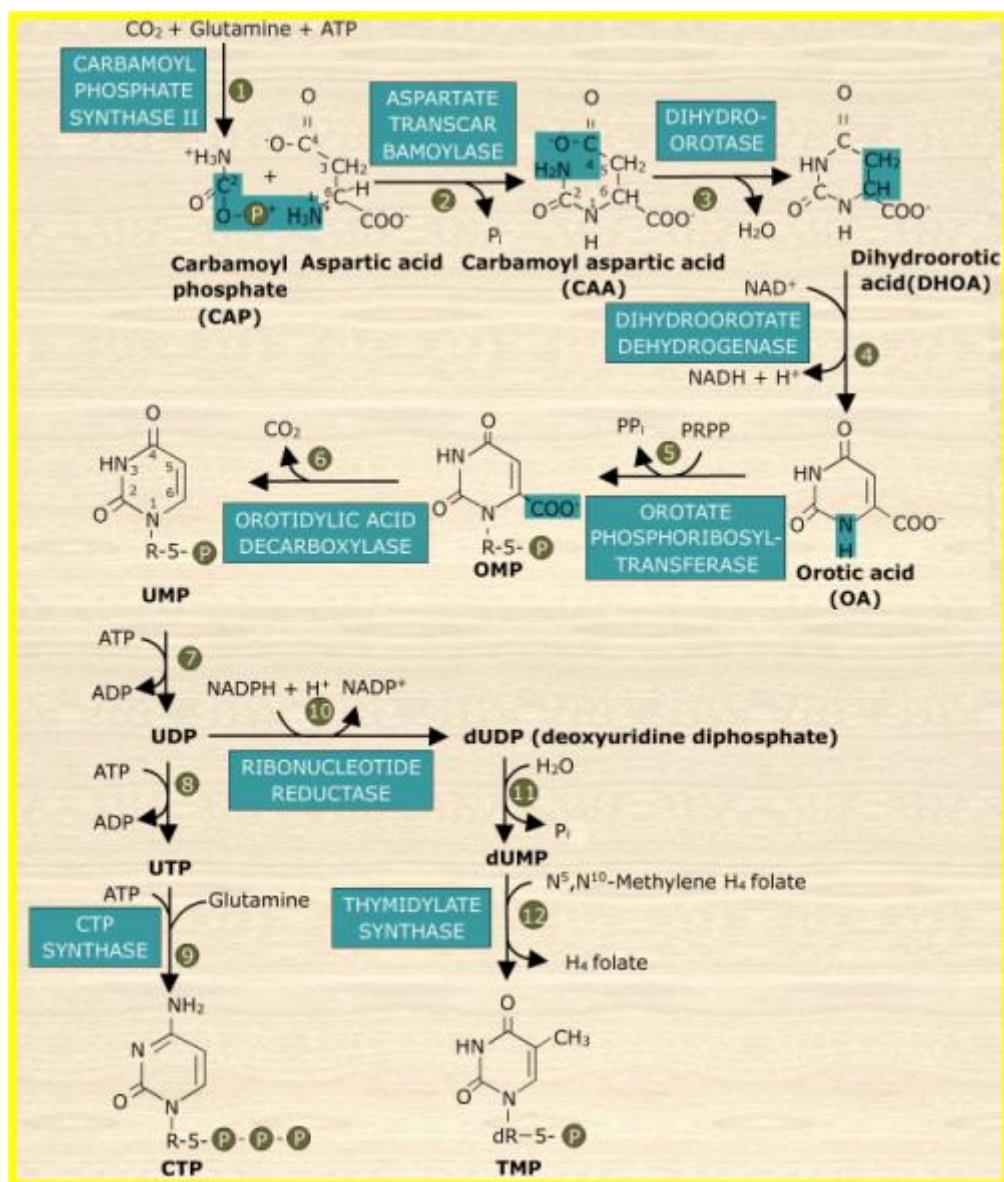
PYRIMIDINE METABOLISM

PYRIMIDINE SYNTHESIS – DE NOVO PATHWAY



Sources for pyrimidine ring

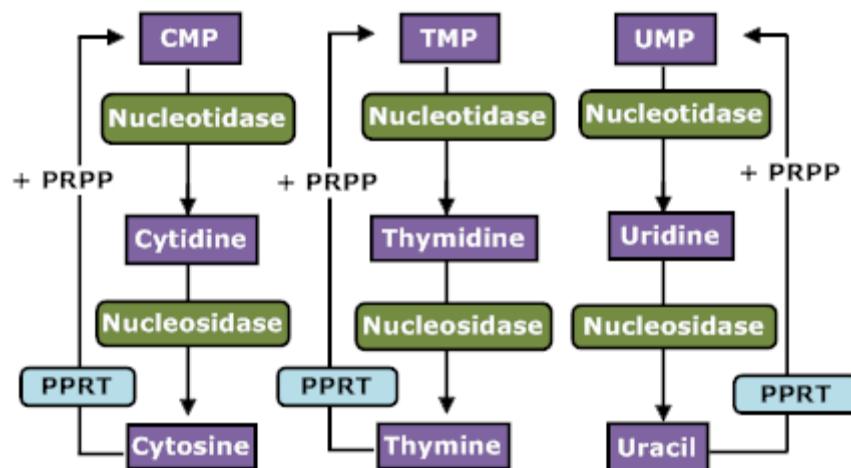
- Carbamoyl phosphate and aspartate are the precursors of pyrimidines ring.
- The first step is the formation of carbamoyl phosphate from bicarbonate, the amide nitrogen of glutamine in the presence of 2 ATP. This reaction is catalyzed by carbamoyl phosphate synthetase II (CPS-II), present in the cytosol. (CPS-I present in mitochondria is involved in urea synthesis).
- Carbamoyl phosphate is combined with aspartic acid to form carbamoyl aspartate catalyzed by the enzyme aspartate transcarbamoylase (ATCase).
- Carbamoyl aspartate cyclizes to form a six membered ring, dihydroorotate by the enzyme dihydroorotase.
- Two hydrogen atoms are removed from the ring by the enzyme dihydroorotate dehydrogenase to form orotic acid.
- The next step is the addition of PRPP to the orotic acid to form orotidine monophosphate (OMP).
- The OMP is then decarboxylated to form UMP mediated by the enzyme OMP decarboxylase.
- UMP is converted to UDP by the enzyme nucleoside monophosphate kinase. It is again phosphorylated to form UTP by nucleoside diphosphate kinase.
- UTP reacts with glutamine in an ATP dependant reaction to form cytidine triphosphate (CTP), which has an amino group on C-4. This conversion utilizes the amide nitrogen of glutamine.
- Similarly, from UDP, by the action of ribonucleotide reductase, dUDP is formed, which is converted to dUMP by phosphorylase.
- Then, thymidylate synthase methylates dUMP into TMP. The methyl donor is N⁵,N¹⁰ - methylene H₄ folate.



PYRIMIDINE SYNTHESIS - SALVAGE PATHWAY

- It is a metabolic pathway that utilizes compounds formed in catabolism for biosynthetic reactions.
- During cellular metabolism and during digestion in animals nucleic acids are degraded to mononucleotides, nucleosides and finally to free bases. Some of the purines and pyrimidines formed in this way are further degraded. But considerable amount is salvaged by reacting with PRPP to reform nucleotides.
- The recycling of bases conserves cellular energy.
- Pyrimidines are converted to nucleotides by the enzyme pyrimidine phosphoribosyltransferase utilizing PRPP as the source of ribose.

SALVAGE PATHWAY FOR PYRIMIDINE SYNTHESIS

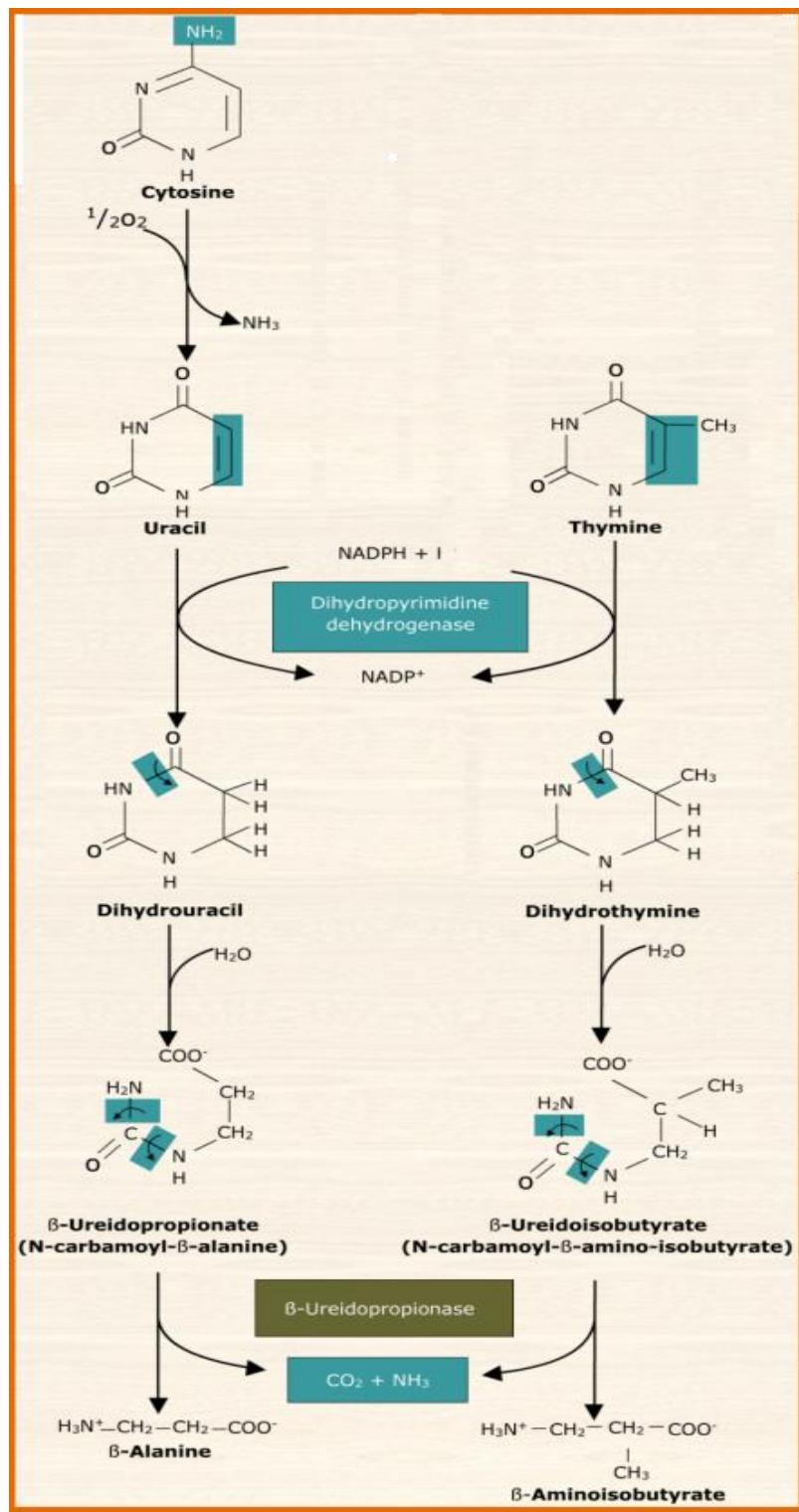


PRPP - 5' Phosphoribosyl pyrophosphate

PPRT - Pyrimidine phosphoribosyl transferase

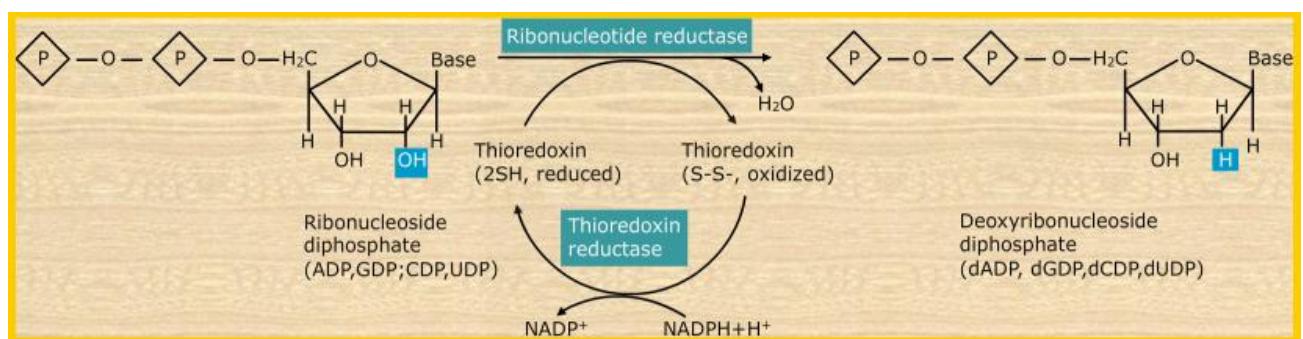
PYRIMIDINE CATABOLISM

- Pyrimidine nucleotides are hydrolyzed to the corresponding nucleosides and Pi by nucleotidases. Then ribose is removed to give the free bases cytosine, uracil and thymine.
- Cytosine is deaminated to produce uracil, which can be converted to dihydrouracil by the enzyme uracil dehydrogenase. The ring is then opened by hydrolysis to yield N-carbamoyl β - alanine. This compound is hydrolyzed to β - alanine, ammonia and CO₂. β - alanine is converted to acetyl Co A.
- Thymine is also metabolized in a series of parallel reactions forming CO₂, ammonia and β - aminoisobutyrate, which is converted to succinyl Co A. The compounds formed are highly water-soluble.
- Ammonia and CO₂ will be used in the synthesis of urea and excreted.
- Overproduction of pyrimidine metabolites does not cause any problem for the body.



FORMATION OF DEOXYRIBONUCLEOTIDES

- Ribonucleotides are converted to deoxyribonucleotides by the replacement of the 2'- hydroxyl group by hydrogen. The substrates are ribonucleotide diphosphates. The enzyme ribonucleotide reductase complex catalyzes this reaction. In this way nucleotide diphosphates such as ADP, GDP, CDP and UDP are converted to their corresponding dADP, dGDP, dCDP and dUDP.
- DNA contains thymine rather than uracil. The immediate precursor is dUMP.
- First the dUTP is hydrolyzed to dUMP.
- $dUTP + H_2O \rightarrow dUMP + PPi$.
- The dUMP is converted to dTMP by thymidylate synthase, which utilizes N^5, N^{10} -methylene tetrahydrofolate as the source of methylene group. The immediate donor of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group is given by a protein called thioredoxin (a protein with two cysteine residues), which after donating hydrogen atoms forms disulfide bonds. Thioredoxin reductase catalyzes the formation of reduced thioredoxin using NADPH as coenzyme.
- The dTMP is phosphorylated to dTTP by the transfer of phosphate from ATP.



ANTIMETABOLITES

Several drugs that are used in the treatment of bacterial infections or in cancer therapy act by inhibiting directly or indirectly the nucleotide metabolism:

- *5-fluorouracil*: It is used in the treatment of cancer; it interferes with the thymidylate synthesis. In the cells salvage pathways convert dUMP to FdUMP, which then binds to the enzyme thymidylate synthase thereby inactivates the enzyme. Inhibition of the enzyme decreases cellular level of thymidine nucleotides. Cells lacking thymine will die.
- *6-mercaptopurine*: It is a hypoxanthine analog, which inhibits purine nucleotide biosynthesis.
- *Azaserine and acivicin*: They are glutamine analogues. Glutamine acts as a nitrogen donor in a number of reactions in the synthesis of nucleotides. The reaction is catalyzed by the enzyme glutamine amidotransferase. The reaction is inhibited by the presence of glutamine analog.
- *Sulfonamide antibiotics*: They are used in the treatment of various infectious diseases. These are structural analog of p-amino benzoic acid, a component of folate. Sulfonamides inhibit competitively the incorporation of p-amino benzoate into folate. Humans are resistant to the action of sulfonamides because folate synthesis does not occur in human cells and preformed folate, a vitamin is required.
- *Trimethoprim*: is an antibiotic that inhibits dihydrofolate reductase in sensitive bacteria. Human dihydrofolate reductase is resistant to the action of trimethoprim.
- *Azathiopurine*: is an immunosuppressive agent used in the treatment of tissue rejection in individuals who have organ transplant. This compound is converted to mercaptoperine by glutathione.
- *Methotrexate*: it is used in the cancer therapy; it is a folate analog, which competitively inhibits the enzyme dihydrofolate reductase. For coenzyme activity, dihydrofolate should be reduced to tetrahydrofolate catalyzed by the enzyme dihydrofolate reductase. Methotrexate prevents the regeneration of tetrahydrofolate.
- *Aminopterin*: also inhibits the activity of dihydrofolate reductase.

DNA SYNTHESIS (DNA REPLICATION)

- Replication means the process of synthesis of DNA. The purpose of DNA replication is to create daughter DNA molecules that are identical to the parental molecule.
- The genetic information contained in DNA should be transferred from parents to the next generation.
- Replication occurs during the “S” phase of the cell cycle and it is the total duplication of DNA.
- The replication is **semi-conservative in nature**. During replication, the parental DNA unwinds and each parental strand acts as a template for the synthesis of new strand. This generates two double stranded daughter DNA molecules. Each daughter molecule of DNA contains one intact parental strand and one newly synthesized strand, which are held together by base pairs.
- Matthew Meselson and Franklin Stahl experimentally proved this semi- conservative mode of replication in 1958.
- The replication is **Bi-directional**, which means that from the site of origin, replication occurs in both directions.
- DNA replication is also **semi-discontinuous**, which means that along one parental template strand that is facing towards the replication fork in 5' to 3' direction, the new daughter DNA will be synthesized continuously (**called as ‘leading strand’**), while along the other parental strand that is running away from the replication fork in 3' to 5' direction, the new daughter DNA will be synthesized dis – continuously or as small fragments (**called as ‘lagging strand’**).
- Both in leading and lagging strands, the direction of DNA synthesis is 5' to 3' only.
- In prokaryotes, DNA replication begins at a single point (**only one origin of replication – ori c**). Both strand serve as template. DNA synthesis proceeds in both strand. In eukaryotes replication begins at multiple sites.
- The complete unit of DNA along with the required enzymes / proteins undergoing replication is called a “**Replicon**”.

CELL CYCLE AND CHARGAFF'S RULE

Cell cycle

- Life of a eukaryotic cell can be defined as a cell cycle. It is the period between the cell formed by the division of its parents to the time it in turn divides. Eukaryotic cells in culture have cell cycle times of 16-24 hours.
- Before a cell can divide it must replicate its DNA.
- The cell cycle consists of four phases.

- The first phase of the cell cycle is G_1 (first gap phase) phase in which the cell prepares to duplicate their chromosomes and synthesizes the required proteins. It is the longest phase.
 - The second phase is S phase where the synthesis of DNA occurs (may take 8-10 hours).
 - The third phase is G_2 phase (the second gap phase) in which the cells prepare to divide.
 - Finally the fourth is M phase in which mitosis occurs. It lasts for one hour. Following mitosis, cells may reenter G_1 phase.
- Some cells *in vivo*, stop dividing completely and are said to be quiescent, locked in a G_0 phase. Upon the appropriate signal, cells in G_0 phase may be stimulated to reenter the cell cycle and divide.

Chargaff's rule

- The base composition of DNA varies from one species to another.
- DNA isolated from different tissues of the same species have the same composition.
- The base composition of DNA in a given species does not change with the organism's age, nutritional status or changing environment.
- In all DNA regardless of species, the number of adenine is equal to the number of thymine and the number of guanine is equal to the number of cytosine. Sum of the purines are equal to sum of pyrimidines $A + G = T + C$.

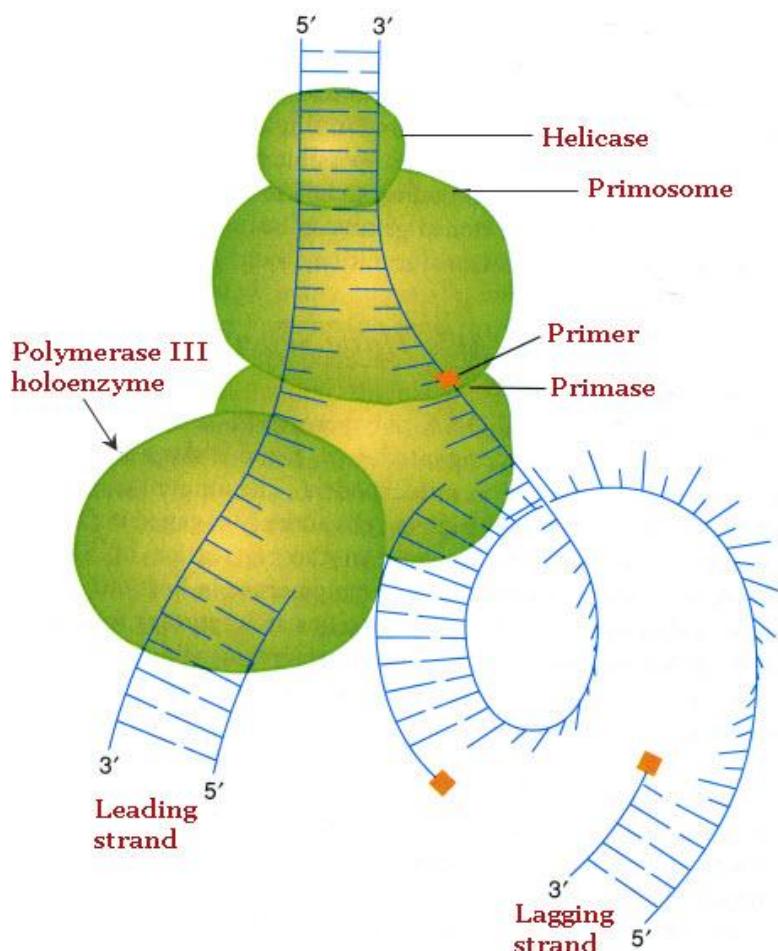
DNA REPLICATION IN PROKARYOTES

Initiation and Creation of the 'Replication bubble' & 'Replication fork'

- Replication of a new DNA starts at specific region known as origin of replication (ori-c). This sequence of DNA is rich in A-T base pairs and is identified by the protein dna A (causes separation of the two strands of DNA).
- DNA Helicases are the group of enzymes that are involved in unwinding of helix from the ori-c (like Zip opener). This enzyme hydrolyzes ATP and utilizes the energy to accomplish the work.
- When the two strands are separated the 'replicon' looks like 'bubble' or 'eye'.
- When one half of the 'replicon' is viewed it looks like a 'Y' shaped structure, which represents the site of active DNA synthesis. This region is called 'replication fork'.
- The unwinding allows each parental DNA strand to act as a template for the synthesis of a new strand.
- To prevent the single stranded DNA strands (ss DNA) from reforming double helix (by forming intrastrand hydrogen bonding), single strand binding proteins (SSB proteins) attach to the

single stranded DNA and keep them apart. They are also known as “DNA helix destabilizing proteins”. They protect the ss DNA from degradation by the action of ‘Nucleases’.

- When the two strands are separated, the entire unreplicated portion of the DNA molecule becomes more twisted on either side of the replication bubble. This in turn causes positive supercoiling of the unreplicated portion (positive supercoiling is formed by twisting the DNA helix in the same direction as the original helix). This process interferes with further unwinding of the double helix.
- Most of the organisms contain a group of enzymes, which are responsible to prevent the extreme supercoiling of the parental helix at the time of unwinding of double helix. These enzymes are called “Topoisomerases”.
- **Topoisomerases I** cleaves a phosphate diester bond in one strand of the helix. The unbroken strand passes through the nick in the broken strand and the phosphodiester bond is replaced or ligated by the same enzyme. This results in unwinding of the two template strands.
- **Topoisomerases II** break both the strands of DNA and then reseals them. “DNA Gyrase”, is a type II topoisomerase found in E.coli (bacteria). This is capable of introducing negative supercoils into circular DNA. This neutralizes the positive supercoil produced during the opening of the double helix.

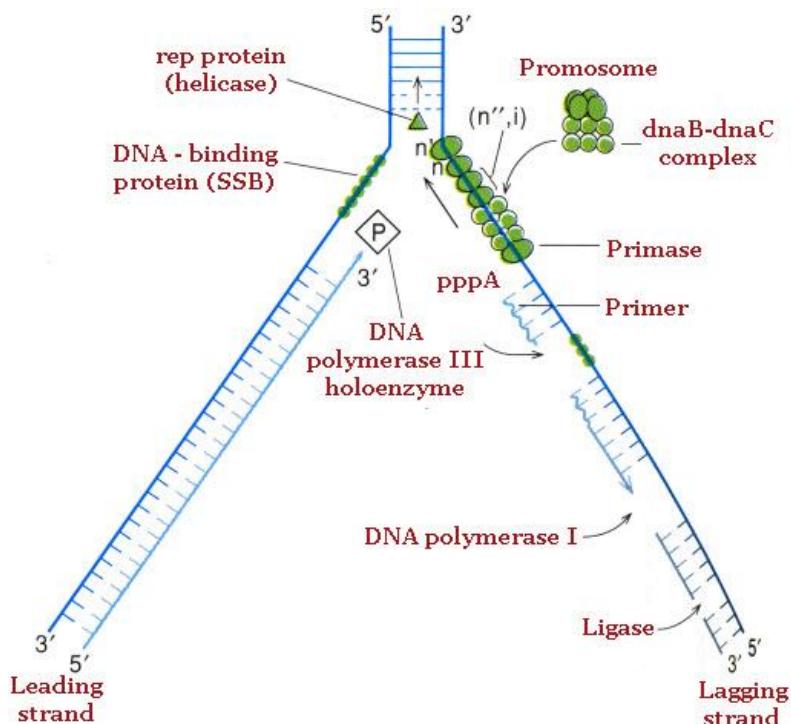


- DNA polymerases are the group of enzymes concerned with DNA synthesis.
- But, they **cannot start DNA synthesis without a primer**. The primer used is a **short piece of RNA** containing around 5-50 ribonucleotides. The RNA primer is synthesized by copying the parental strand of DNA by an enzyme called **primase**, which is an RNA polymerase. The primase along with the RNA primer is called '**Primosome**'.
- DNA polymerase then extends the RNA primer made by primase by adding the nucleotide to the 3' hydroxyl group of the RNA primer.
- Only one RNA primer is required along the 'leading strand', while several RNA primers are made along the length of the 'lagging strand'.

DNA synthesis and action of DNA polymerases

- DNA synthesis occurs in 5' to 3' direction only (in both the strands).
- Prokaryotes have three DNA polymerases: DNA Polymerase I (DNA Pol I), DNA Polymerase II (DNA Pol II) and DNA Polymerase III (DNA Pol III).
- DNA Polymerase III catalyzes the synthesis of new DNA molecules.
- Each RNA primer synthesized by the primase is extended in the 5'→3' direction by DNA polymerase III, until it reaches the 5' end of the next RNA primer. The enzyme dissociates from the DNA, leaving a nick or break at the DNA/RNA junction.
- **DNA Pol III catalyzes DNA synthesis in 5' to 3' direction.** It catalyses the stepwise addition of deoxyribonucleotide to the 3' OH end of a DNA chain creating 3'→5' phosphodiester bond and releases pyrophosphate.
- During polymerization if a wrong nucleotide is incorporated, it is removed using the 3'—5' exonuclease activity of DNA polymerase III and correct base is incorporated. This is known as "proof reading activity" (It is estimated that one base in five billion bases may be inserted incorrectly).
- The substrates are the four deoxyribonucleoside triphosphates viz. dATP, dCTP, dGTP & dTTP.
- DNA polymerase reads the template strand and adds a nucleotide to a free 3'- hydroxyl group and synthesizes the DNA in the 5' to 3' direction.
- As DNA synthesis occurs only in the 5' to 3' direction, both the DNA strands are copied simultaneously. One in the 5' to 3' direction towards the replication fork and one in the 5' to 3' direction away from the replication fork.
- When the parental strand that runs 3'→5' direction towards the replication fork is copied, the resulting strand is synthesized continuously and is known as **leading** strand.
- When the parental strand that runs 3'→5' away from the replication fork is copied the resulting strand is synthesized discontinuously and is known as **lagging** strand.
- When the DNA polymerase copies the parental strand running away from the replication fork, several short fragments are formed (lagging strand). These small fragments are called **Okazaki** fragments, which are about 50-250 deoxyribonucleotides in length.

- DNA Pol III continues the synthesis, until it reaches the 5' end of the next RNA primer. The enzyme dissociates from the DNA, leaving a nick or break at the DNA / RNA junction.
- **DNA Pol I** first removes the RNA primer by its 5'→3' exonuclease activity. Then it attaches to the nick and adds nucleotide to the 3'OH in the 5'→3' direction. (DNA Pol III does not have a 5'→3' exonuclease activity & hence, it cannot remove RNA primer).
- In the final step, the Okazaki fragments should be joined together by a phosphodiester linkage. An enzyme called **DNA ligase** carries this work. Thus **DNA ligase** seals the gap of between the DNA synthesized by the DNA Pol III and DNA Pol I.
- Now the DNA replication is complete so that from one parental DNA strands two daughter DNA strands are produced in semi-conservative manner.
- **DNA polymerase II** is considered to take part in DNA repair.



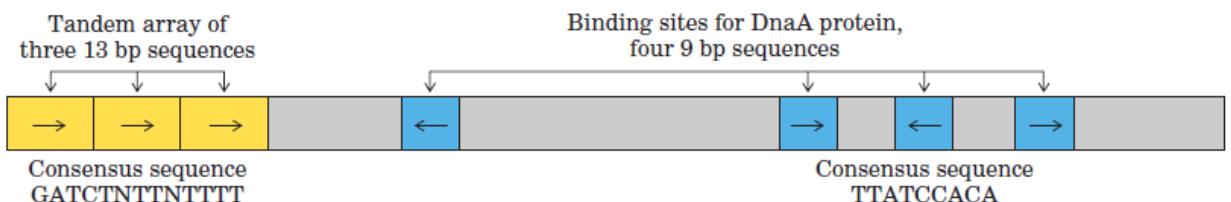
TYPES OF DNA POLYMERASES

PARAMETERS	DNA polymerase		
	I	II	III
3'→5' Exonuclease (Proof reading)	Yes	Yes	Yes
5'→3' Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16 - 20	40	250 – 1,000
Processivity (nucleotides added before polymerase dissociates)	3 - 200	1,500	≥ 500,000

- DNA replication can also be described under 3 stages viz., Initiation, Elongation and Termination, for better understanding. These processes (as it occurs in E.Coli) are also described separately, in the following sections.

INITIATION OF DNA REPLICATION

- The E.Coli replication origin, OriC consists of 245bp; it bears DNA sequence elements that are highly conserved among bacterial replication origins.
- The general arrangement of the conserved sequences is illustrated in the diagram. The key sequences of interest here are two series of short repeats: i.e. three repeats of a 13 bp sequence and four repeats of a 9bp sequence.
- At least nine different enzymes or proteins participate in the initiation phase of replication. They open the DNA helix at the origin and establish a prepriming complex for subsequent reactions.



- The crucial component in the initiation process is the DnaA protein. A single complex of four to five DnaA protein molecules binds to the four 9bp repeats in the origin, then recognizes and successively denatures the DNA in the region of the three 13bp repeats, which are rich in A=T pairs.
- The process requires ATP and the bacterial histonelike protein HU.
- The DnaC protein then loads the DnaB protein onto the unwound region. Two ring shaped hexamers of DnaB, one loaded onto each DNA strand, act as helicases, unwinding the DNA bidirectionally and creating two potential replication forks.
- Many molecules of SSB (Single stranded DNA binding protein) bind cooperatively to single-stranded DNA, stabilizing the separated strands and preventing renaturation, while gyrase (Topoisomerase II) relieves the topological stress produced by the DnaB helicase.

Regulation

- Initiation is the only phase of DNA replication that is known to be regulated, and it is regulated such that replication occurs only once in each cell cycle. The mechanisms of regulation is not yet well understood, but genetic and biochemical studies have provided a few insights.
- The timing of replication initiation is affected by DNA methylation and interactions with the bacterial plasma membrane.

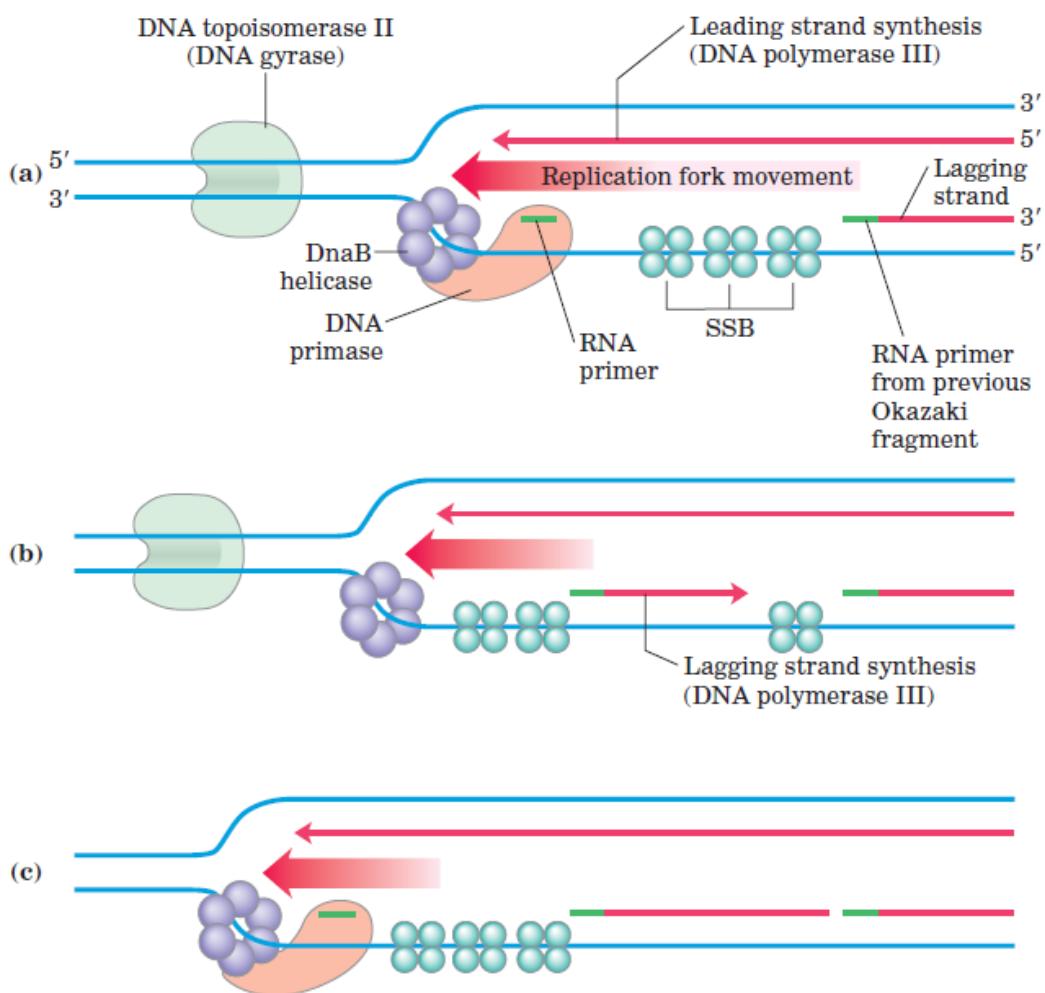
- The OriC DNA is methylated by the Dam methylase, which methylates the N⁶ position of adenine within the palindromic sequences (5') GATC. (Dam is not a biochemical expletive; it stands for DNA adenine methylation.) The oriC region of E.coli is highly enriched in GATC sequences - it has 11 of them in its 245 bp.
- Immediately after replication, the DNA is hemimethylated: the parent strands have methylated oriC sequences but the newly synthesized strands do not. The hemimethylated oriC sequences are now sequestered for a period by interaction with the plasma membrane (the mechanism is unknown).
- After a time, oriC is released from the plasma membrane, and it must be fully methylated by Dam methylase before it can again bind DnaA.
- Regulation of initiation also involves the slow hydrolysis of ATP by DnaA protein, which cycles the protein between active (with bound ATP) and inactive (with bound ADP) forms on a timescale of 20 to 40 minutes.

Protein	M _r	Number of Subunits	Function
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5') GATC sequences at oriC

ELONGATION OF DNA REPLICATION

- The elongation phase of replication includes two distinct but related operations: leading strand synthesis and lagging strand synthesis. Several enzymes at the replication fork are important to the synthesis of both strands.
- Parent DNA is first unwound by DNA helicases, and the resulting topological stress is relieved by topoisomerases. Each separated strand is then stabilized by SSB. From this point, synthesis of leading and lagging strands is sharply different.
- Leading strand synthesis, the more straightforward of the two, begins with the synthesis by primase (DnaG protein) of a short (10 to 60 nucleotide) RNA primer at the replication origin.

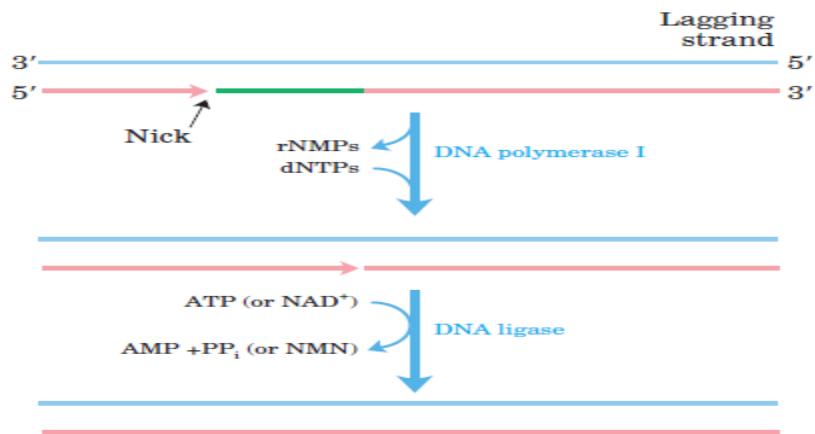
- Deoxyribonucleotides are added to the primer by DNA polymerase III. Leading strand synthesis then proceeds continuously, keeping pace with the unwinding of DNA at the replication fork.
- Lagging strand synthesis, is accomplished in short Okazaki fragments. First, an RNA primer is synthesized by primase and, as in leading strand synthesis, DNA polymerase III binds to the RNA primer and adds deoxyribonucleotides.



- On this level, the synthesis of each Okazaki fragment seems straightforward, but the reality is quite complex.
- The complexity lies in the coordination of leading and lagging strand synthesis: both strands are produced by a single asymmetric DNA polymerase III dimer, which is accomplished by looping the DNA of the lagging strand as shown in the diagram, bringing together the two points of polymerization.
- The synthesis of Okazaki fragments on the lagging strand entails some elegant enzymatic choreography. The DnaB helicase and DnaG primase constitute a functional unit within the replication complex, the primosome.
- DNA polymerase III uses one set of its core subunits (the core polymerase) to synthesize the leading strand continuously, while the other set of core subunits cycles from one Okazaki fragment to the next on the looped lagging strand.

- The DnaB helicase unwinds the DNA at the replication fork as it travels along the lagging strand template in the 5' → 3' direction.
- DNA primase occasionally associates with DnaB helicase and synthesizes a short RNA primer.
- A new β sliding clamp is then positioned at the primer by the clamp-loading complex of DNA polymerase III. When synthesis of an Okazaki fragment has been completed, replication halts, and the core subunits of DNA polymerase III dissociate from their β sliding clamp (and from the completed Okazaki fragment) and associate with the new clamp.
- This initiates synthesis of a new Okazaki fragment. As noted earlier, the entire complex responsible for coordinated DNA synthesis at a replication fork is a **replisome**.
- The replisome promotes rapid DNA synthesis adding ≈ 1,000 nucleotides/s to each strand (leading and lagging). Once an Okazaki fragment has been completed, its RNA primer is removed and replaced with DNA by DNA polymerase I and the remaining nick is sealed by DNA ligase.
- DNA ligase catalyzes the formation of phosphodiester bond between a 3' hydroxyl end of one DNA strand and a 5' phosphate at the end of another strand. The phosphate must be activated by adenyllylation. DNA ligases isolated from viruses and eukaryotes use ATP for this purpose. DNA ligases from bacteria are unusual, in that they generally use NAD⁺ - a cofactor that normally functions in hydride transfer reactions as the source of the AMP activating group.
- DNA ligase is another enzyme of DNA metabolism that has become an important reagent in recombinant DNA experiments.

Protein	M _r	Number of Subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	791,500	17	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps; excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling



TERMINATION OF DNA REPLICATION

- Eventually, the two replication forks of the circular E.coli chromosome meet at a terminus region containing multiple copies of a 20bp sequence called Ter (for terminus).
- The Ter sequences are arranged on the chromosome to create a sort of trap that a replication fork can enter but cannot leave.
- The Ter sequences function as binding sites for a protein called Tus (terminus utilization substance). The Tus-Ter complex can arrest a replication fork from only one direction.
- Only one Tus-Ter complex functions per replication cycle - the complex first encountered by either replication fork. Given that opposing replication forks generally halt when they collide. Ter sequences do not seem essential, but they may prevent overreplication by one replication fork in the event that the other is delayed or halted by an encounter with DNA damage, or some other obstacle.
- So, when either replication fork encounters a functional Tus-Ter complex, it halts; the other fork halts when it meets the first (arrested) fork. The final few hundred base pairs of DNA between these large protein complexes are then replicated (yet unknown mechanism), completing two topologically interlinked (catenated) circular chromosomes.
- DNA circles linked in this way are known as **catenanes**. Separation of the catenated circles in E.Coli requires topoisomerase IV (a type II topoisomerase). The separated chromosomes then segregate into daughter cells at cell division.
- The terminal phase of replication of other circular chromosomes, including many of the DNA viruses that infect eukaryotic cells, is similar.

DNA REPLICATION IN EUKARYOTES

DNA Replication in eukaryotes (Differences from prokaryotes)

- It has multiple sites of origin.
- In eukaryotes, DNA is replicated by **five DNA polymerases - $\alpha, \beta, \gamma, \delta$ and ϵ**
- **DNA Pol α** is involved in the synthesis of RNA primer in both leading and lagging strands. **DNA Pol β** is involved in the repair of DNA and **DNA Pol γ** is involved in mitochondrial DNA replication. **DNA Pol δ** is concerned with DNA synthesis in leading strand and also does proof reading, while **DNA Pol ϵ** is concerned with DNA synthesis in lagging strand and also does proof reading.
- Along with DNA, the basic proteins “Histones” are also synthesized. Unlike the semi-conservative mode of DNA replication, the newly synthesized histones are transferred to one daughter cell while the original set of histones is conserved in another daughter cell (conservative mode).

RNA SYNTHESIS (TRANSCRIPTION)

- Transcription is a process by which RNA is synthesized from DNA.
- There are 3 major classes of RNA. They are ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA).
- All of them take part in protein synthesis.
- Transcription is ‘**selective**’ i.e only particular part of DNA gets transcribed and not the whole genome (total DNA); this segment of DNA is called ‘**gene**’.
- In any gene, one strand of DNA is acting as a template for transcription. RNA is produced using the template strand. The template strand is also known as non-coding strand or antisense (-) strand.
- The non-template strand also known as coding strand or sense (+) contains the sequences present in mRNA.
- The sequence of nucleotides in mRNA is complementary to the nucleotide sequence of template DNA.
- The bases T, A, G and C in the DNA template will bind with bases A, U, C and G respectively in RNA.
- DNA is transcribed into RNA by the enzyme RNA polymerase. The RNA molecule synthesized is known as transcript. The DNA template is copied in the $3' \rightarrow 5'$ direction and the RNA chain grows in $5' \rightarrow 3'$ direction.
- RNA polymerase can initiate the synthesis of a new strand without a primer.
- Ribonucleotide triphosphates (ATP, GTP, UTP and CTP) serve as precursors for the RNA chain.

Gene notation

- It refers to description of the position of nucleotides in a gene.
- The nucleotide in the DNA template at which transcription begins is designated as +1
- The transcription proceeds in the downstream direction. The nucleotides in the transcribed DNA are given successive positive numbers.
- The nucleotides that lie to the left of this site are called the upstream sequence and are identified by negative numbers.

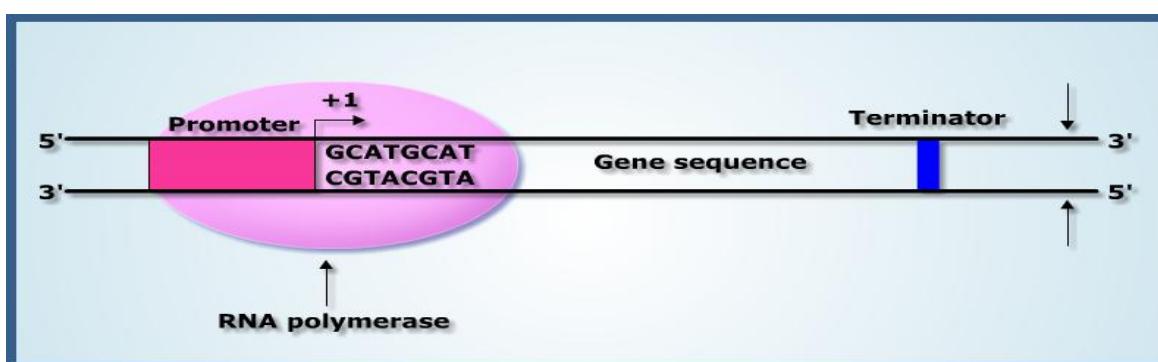
TRANSCRIPTION IN PROKARYOTES

Synthesis of RNA in bacteria

- The process of transcription in prokaryotes can be divided into three phases.
 - Initiation
 - Elongation
 - Termination.
- In E.coli, all genes are transcribed by the same RNA polymerase, which contain six subunits. Two α , one β , one β' and one ω , which form the core enzyme and a sixth subunit σ (sigma) factor which is required for the initiation of RNA synthesis. This complete enzyme is called holoenzyme.

INITIATION OF TRANSCRIPTION

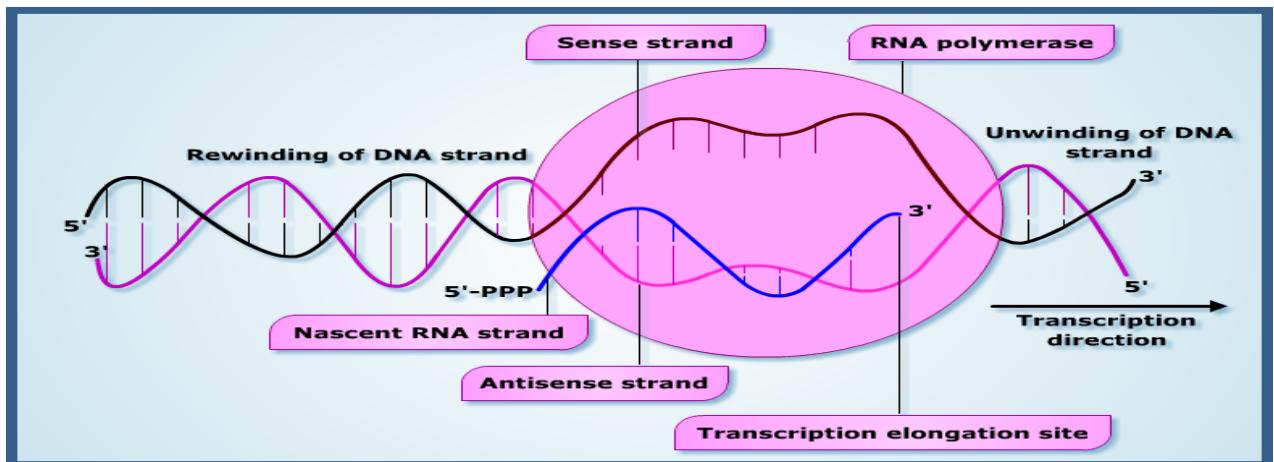
- This involves the binding of RNA polymerase to the specific region on the DNA known as 'promoter' region.
- Promoter contains two important sequences (known as consensus sequences) one that lies just -10 base pairs upstream from the start point of transcription, which contains TATAAT (called Pribnow box).
- The second sequence TTGACA is usually located upstream from the Pribnow box, about 35 nucleotides from the start site (-35 from the start point of transcription).



- A consensus sequence consists of most commonly found sequences of bases in a given region of the entire DNA tested.
- To start transcription, RNA polymerase (holoenzyme) binds to a promoter sequence, which unwinds or partially separates the two strands of DNA. This enables one strand to act as a template for the synthesis of RNA molecule.
- The σ (sigma) factor helps in the recognition of promoter sequence.
- The separation of two stands of DNA is helped by A-T rich sequence of the Pribnow box (there are only two hydrogen bonds between the bases adenine and thymine; so it is easy to separate the two strands at this point).

ELONGATION OF TRANSCRIPTION

- As the polymerase moves along the DNA the next region of the double helix unwinds and the RNA chain grows.
- When the RNA chain is about ten bases in length, the σ factor is released from the RNA polymerase and the rest of the enzyme moves along the DNA adding ribonucleotides to the 3' end of the growing RNA molecule.
- Another accessory protein called 'Nus A protein' is required, which binds to the RNA Pol core enzyme and helps in elongation of RNA chain.
- Transcription continues until a termination signal is reached.



TERMINATION OF TRANSCRIPTION

- E.coli has specific sequences called 'termination sequences' or 'terminators' at the end of the gene that causes RNA polymerase to stop transcribing DNA.
- There are two types of termination
 - Rho dependent termination
 - Rho independant termination.

- **Rho dependant termination:** The terminator sequences are recognized by a protein known as rho (ρ), which binds to the growing RNA and RNA Pol. At bound state it acts as ATPase (RNA-DNA helicase) and terminates transcription, releasing RNA, Nus A from the transcription complexes and DNA double helix is reformed.
- **Rho independent termination:** The terminator sequence in DNA template has palindrome sequences, which form **hairpin loops in RNA** upon transcription. This causes distortion in the overall dimensions so that the DNA strand can no longer bind to the RNA and RNA cannot be accommodated inside transcription unit. Hence, the RNA molecule & Nus A proteins are released, transcription terminates and DNA double helix gets reformed. (The termination sequences consist of two regions rich in the bases 'G' and 'C'. This sequence is followed by a stretch of 'A' base. When 'GC' rich regions (palindrome) are transcribed they form a hairpin loop in RNA that precedes three U residues).

TRANSCRIPTION IN EUKARYOTES AND A COMPARISON WITH PROKARYOTES

Synthesis of RNA in eukaryotes (Differences from prokaryotes)

RNA polymerase

- Eukaryotes have three types of RNA polymerases and these are responsible for the synthesis of different classes of RNAs.
- RNA polymerase I transcribe genes that code for most of the ribosomal RNA.
- All messenger RNAs are synthesized using RNA polymerase II.
- Transfer RNAs are synthesized by RNA polymerase III.
- The chemical reaction catalyzed by these three RNA polymerases (formation of phosphodiester bond) is the same in eukaryotes and bacteria.

Promoter sequences

- A sequence of DNA nucleotides that is almost identical to that of Pribnow box is found about – 25 nucleotides upstream of the initial base of the start site. This consensus sequence is called the '**TATA or Hogness box**'.
- Between 70 and 80 nucleotides upstream from the transcription start site is a second consensus sequence known as the '**CAAT**' box.
- One or both of these sequences can serve as recognition site in eukaryotic promoters.

Post –transcriptional modifications

- In prokaryotes, the genes and mRNA, which are coding for the proteins are continuous and can be translated as such (i.e. introns are absent). So, the transcripts formed are mature to take part in the translation process. And generally, the processes of transcription and translation are continuous and simultaneous in prokaryotes.

- But in eukaryotes, the primary transcript of mRNA (also called heterogeneous RNA –hnRNA) formed is immature and undergoes some modifications called '**Post-transcriptional modifications**' to become functionally mature.
- There are usually three modifications, which occurs in **mRNA**.
- They are: 1.Capping 2. poly 'A' tail and 3. Splicing.

1. Capping: The 5' terminal triphosphate is modified by the addition of a guanosine via 5'-5' phosphodiester bond. This sequence is subsequently methylated to form a 7-methylguanosine cap. This helps in protecting the mRNA from undergoing degradation, when it travels from nucleus to cytosol (ribosomes).

2. Poly 'A' tail: The 3' end of nearly all eukaryotic mRNA are modified by the addition of a long stretch of adenosine residues, the poly A tail by the enzyme polyadenylate polymerase. This helps in directing the mRNA from the nucleus to the ribosomes.

3. Splicing: The coding regions in mRNA called exons are separated by non -coding / intervening sequences called introns. Introns are removed by the process called RNA splicing. Here, the introns are removed and exons are joined together.

- In tRNA the post-transcriptional modifications include,
 1. Modification of nucleotides, which includes conversion of uridine to pseudouridine (ϕ), ribothymidine and dihydrouridine.
 2. Addition of the sequence CCA to the 3' end is catalyzed by nucleotidyl transferase.
- The rRNA is initially synthesized as **pre-rRNA** and then undergoes certain post-transcriptional modifications to become **functional rRNA**.

INHIBITORS OF TRANSCRIPTION

Some antibiotics inhibit the process of transcription and examples include:

- **Actinomycin D:** (Dactinomycin) this is synthesized by Streptomyces. It binds to the DNA template and blocks the movement of RNA Pol. This was earlier used for treating cancer.
- **Rifampin:** this binds to the β -subunit of prokaryotic RNA pol and inhibits its activity. This is used for the treatment tuberculosis and leprosy.
- **α – amainitin:** this is a toxin secreted by the mushroom Amanita phalloides. This binds with the RNA Pol II of eukaryotes and inhibits transcription.

MINERAL METABOLISM

- Animals need inorganic elements or minerals for their normal life process.
- Minerals are solid, crystalline chemical elements, not decomposed or synthesized by ordinary chemical reactions.
- More than 20 minerals are essential in animal nutrition. Of these seven are bulk / macro minerals (Ca^{2+} , Mg^{2+} , K^+ , Na^+ , S , Cl^- and PO_4^{3-}) and the rest are referred to as trace minerals.
- The minerals that are required in the diet may be divided into two groups such as macro-minerals and trace minerals, based on the level of daily requirement.
- Requirement of macro-minerals is more than 100 mg/day and requirement of trace minerals are few mg/day.
- Mineral elements can also be classified as
 - Cations – Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Fe^{2+} , Mn^{2+} and Zn^{2+}
 - Anions - Cl^- , I^- and PO_4^{3-}
- Unlike other nutrients, mineral elements cannot be synthesized by living organisms.
- Generally they act as structural components, constituents of body fluids and tissues, as electrolytes, co-factors in enzyme activity and in the maintenance of acid-base balance, osmotic pressure, excitability of muscle and nerves, improved feed intake, milk production and milk composition.
- Mineral requirements are highly dependent on the level of productivity. High producing dairy cows requires much more dietary Ca and P than low yielding cows, because milk is rich in those elements.
- Zn^{2+} requirements for spermatogenesis and testicular development in male sheep are higher than those needed for normal growth.
- Requirement of minerals also vary with breed and individual animal variation.
- Adequate intake of feed by animals is essential in meeting mineral requirements.

CALCIUM

- Calcium (Ca) is the mineral, which occurs, in the largest quantities-around 1200g in animal body, mainly in teeth and bone.
- Calcium is generally deficient in grains and abundant in most forage.
- The bulk of calcium (98%) in the bodies of adult animals is found in the bone tissue in the form of Hydroxyapatite ($3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$) crystals. Remaining 2% is present in blood.
- Calcium is present in blood serum as two main fractions- one capable of diffusing (65%) and another is incapable (35%). The diffusible part is present as ionic calcium and bicarbonate, phosphate and citrate complexes, which is physiologically active.
- The non-diffusible calcium is present in bound form with serum albumin and globulin.
- The blood calcium level is maintained by three hormones viz., parathormone, calcitonin and Vit. D3 (calcitriol).

Biochemical functions

- Required for the general growth of bone and teeth.
- Calcium ion is required for the function of nerves and muscle contraction.
- Calcium ions activate some of the enzyme processes. Some of the enzymes of blood clotting process require calcium.
- Milk and eggshell formation require calcium.
- Some of the hormones require calcium as second messenger.
- Calcium ion promotes the secretion of acetylcholine, helping the nerve conduction.

Deficiency diseases

- Deficiency of calcium results in rickets in young animals. Characteristic symptoms of this disease include stunted growth, impaired growth, distorted spine and lameness.
- Deficiency of calcium in adults results in osteomalacia (demineralization of bones without making up for the losses) and osteoporosis (bone porosity produced by resorption of both mineral and organic components).
- In lactating cows, Ca deficiency results in milk fever.
- In laying hens, soft-shelled eggs are produced. Soft beak and bone, retarded growth are also seen in Ca^{2+} deficiency.

Absorption of calcium is influenced by many factors

- **pH:** Alkaline pH is not suitable for absorption.
- **Ratio of Ca to P in the diet** – The optimum Ca: P ratio for the better absorption is 1.3:1 to 1.5:1. Higher the phosphate content, lower the calcium absorption.
- **Vitamin D:** Presence of vitamin D₃ helps better absorption of Calcium.
- Presence of **free fatty acid** reacts with calcium and forms insoluble calcium soaps, which prevents calcium transport.
- If **anions** (negatively charged ions), which bind or precipitate calcium (oxalates, phytates, phosphates and possible sulphates) are present in excess, they may interfere with the absorption of calcium in the intestine.

PHOSPHORUS

Phosphorus (P)

- It is an important constituent of bone and teeth.
- 80% of the total phosphorus is in the combined form with calcium.
- It is a constituent of high-energy compounds such as ATP and creatine phosphate.
- It is required for the phosphorylation of some carbohydrates and their metabolism in the body.
- Some enzymes are active if they are phosphorylated.

- Phosphorus is needed for the synthesis of phospholipids, which are the constituents of the cell membranes.
- It is needed for the formation of DNA and RNA.
- Some phosphoproteins and coenzymes like NAD^+ , NADP^+ , FMN, FAD and pyridoxal phosphate, have phosphorus as their constituents.
- Phosphate buffer is one of the buffers of plasma.
- Phosphorus is needed for normal milk secretion, egg formation and efficient feed utilization.

Absorption

- Absorption of phosphorus is similar to calcium.
- Vitamin D₃ promotes the absorption of phosphorus along with calcium in the intestine.

Deficiency

- In hyperparathyroidism, there is a reduction in the serum phosphate level and an increase in urine phosphate level.
- In hypoparathyroidism, serum phosphate level is increased.
- As phosphorus is needed for the formation of bone, a deficiency can cause rickets or osteomalacia.
- Phosphorus deficiency can cause muscular weakness, low fertility rate, reduced milk yield in cow.
- Phosphorus deficiency is more common in cattle than in sheep or goat.
- Deficiency causes abnormal appetite in cattle - Animals chew wood, bones and other foreign materials. This symptom is known as 'Pica'.

Sources

- Milk, grains, bone meal, meat and leafy vegetables.

IRON

- Iron (Fe), is essential for a number of physiological and metabolic processes.
- Iron is a transition metal with an atomic weight of 56. It has two stable oxidation states (+2 and +3).
- It is present in several enzymes responsible for electron transport (cytochromes), in which, oxidation occurs with the production of adenosine triphosphate; for the activation of oxidases and oxygenases; and for oxygen transport (hemoglobin and myoglobin).
- It plays an important role in the citric acid cycle.
- Lactoferrin is an iron binding protein in milk.
- Ascorbic acid maintains iron in the reduced state in the intestine. In the intestinal mucosal cells, iron is bound by the iron storage protein called ferritin, which regulates the amount of iron entering the bloodstream.

- The iron which crosses into the capillaries is transported by binding to plasma protein called transferrin.

Deficiency

- It occurs mainly in young animals, since the iron content of milk is low.
- The main symptoms of iron deficiency in all species of animals is microcytic and hypochromic anemia. This is due to inadequate synthesis of hemoglobin. In addition, the other changes include lower weight gain, listlessness, labored breathing, reduced appetite and decreased resistance to infection.
- Anemia in piglets: Due to quick growth, they are unable to meet out the demand for iron, which leads to anemic state.
- Iron deficient anemia is not common in lambs and calves.

COBALT

- The only known functions of cobalt (Co) are its participation in metabolism as a component of vitamin B₁₂.
- Most of the cobalamines occur as two co-enzymatically active forms,
 - Deoxyadenosylcobalamin and
 - Methylcobalamin.
- Cyanocobalamin is converted within cells to either methylcobalamin, a coenzyme for methyl transferase or deoxyadenosylcobalamin, the co- enzyme for mutase.
- Most reactions of vitamin B₁₂ involve in transfer and synthesis of one-carbon units. E.g. methyl group.
- The other functions are:
 - In the synthesis of purine and pyrimidines.
 - Formation of proteins from amino acids.
 - In carbohydrate (in the synthesis of glucose from propionic acid) and fat metabolism.
 - It promotes red blood cell synthesis and maintains the integrity of nerve cells.

Deficiency

- The result of Vit B₁₂ deficiency is megaloblastic anaemia, pernicious anaemia and neurological lesions.
- Deficiency is characterized by listlessness and called as ‘pining’, ‘salt sick’, ‘bush sickness’ or ‘wasting disease’.

SELENIUM

- Selenium (Se), is closely linked to Vitamin-E. Both the nutrients protect biological membranes from oxidative degeneration. Lack of these nutrients result in tissue breakdown.
- Selenium is an essential constituent of glutathione peroxidase, which protects cellular and sub-cellular membranes from oxidative damage by destroying peroxides.

- Selenium spares vitamin-E. It reduces the amount of vitamin-E required to maintain the integrity of lipid membranes.
- Vitamin-E also reduces selenium requirement.
- Other functions are
 - It plays a specific role in prostaglandin synthesis and fatty acid metabolism.
 - It is needed for immune response.
 - In RNA synthesis.
 - For wool growth in lambs.
 - In the eye, it is involved in the photochemical reactions of light perception.
 - Selenium supplementation has been found to cure myopathies in sheep and cattle and exudative diathesis in chicks.

VITAMINS

FAT SOLUBLE VITAMINS

- Vitamins are organic nutrients needed daily in very small amount to perform a variety of biochemical functions in the body.
- The body cannot synthesize most of the vitamins; they must be obtained from the diet.
- Vitamins are essential for growth and health. Absence or deficiency of vitamins creates specific disorders.
- Most of the co-enzymes are synthesized from vitamins. The symptoms of vitamin deficiencies reflect the loss of specific enzymes activities depending on the co-enzyme form of the vitamin.
- Synthetic vitamins are nutritionally equivalent to naturally occurring vitamins.
- In the digestive process vitamins interact with other vitamins and nutrients to enhance absorption.
- Vitamins can function as coenzymes, i.e., they can work with enzymes to speed up body's biochemical reactions.
- Vitamins do not provide energy. They help to release energy from the biological reactions during metabolism.
- Vitamins are not structural component of the body.

STORAGE OF VITAMINS AND FAT SOLUBLE VITAMINS

How vitamins are stored?

- Fat-soluble vitamins are stored in body fat and organs, especially the liver. Hence, it can delay deficiency for several months. Impaired absorption of fats also leads to the impaired absorption of fat- soluble vitamins.
- Water-soluble vitamins are not stored in the body. Hence, vitamin deficiency appears within a few weeks after dietary deprivation.

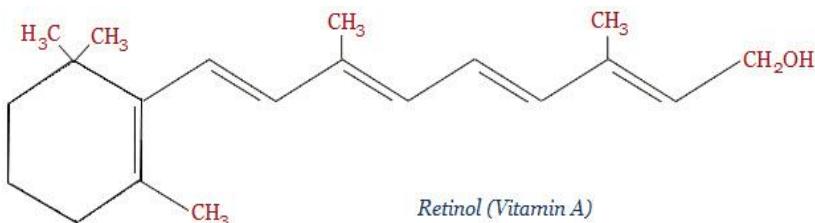
Fat soluble vitamins

- The fat-soluble vitamins are apolar hydrophobic molecules, which are isoprene (2-methyl-1,3-butadiene) derivatives.
- In nature, many essential oils and products of plants contain isoprene in a modified form (isoprenoid). Many number of isoprenoid units condense among themselves to form a polyisoprenoid unit. Most of the lipid soluble vitamins contain such a polyisoprenoid unit as their chemical structure.

VITAMIN A

- Vitamin A, as such is present only in foods of animal origin. However, its provitamins "carotenes" are found in plants.

Chemistry



- β -carotenes present in the plant food are a polyisoprenoid compound containing 2 molecules of retinaldehyde attached tail to tail manner.
- These β -carotenes are cleaved in the intestine to produce 2 molecules of retinaldehyde (retinal). Then, the retinal is converted into retinol and retinoic acid, by reduction and oxidation respectively.
- Thus, retinol, retinoic acid and retinal are all referred as vitamin A. Retinol has the full vitamin A activity.
- Vitamin A or retinol contains a β -ionone ring. The side chain contains two isoprene units and one alcohol group.
- Vitamin A is generic term referring to all the compounds from animal sources that exhibit the biological activity of vitamin A. It is stored in the liver as retinyl ester.
- For transport to tissues, it is hydrolysed and retinol is bound to apo-RBP (retinol binding protein). The resulting holo-RBP is processed in the golgi apparatus and secreted into the plasma.
- Retinoic acid is transported in plasma bound to albumin.

Biological functions

- Enables the eye to adjust to changes in light (formation of rhodopsin in the retina).
- Essential for the integrity of the mucous secreting epithelial cells.
- Aids in the synthesis of ground substances (mucopolysaccharides) of teeth, bones, cartilages, tendons and connective tissues.
- It is also used for the synthesis of mucoproteins and glycoproteins.
- Aids in the reproductive processes.
- Promotes the synthesis of glycogen in liver.
- Regulates fat metabolism in the formation of cholesterol.
- Acting as an antioxidant; hence, preventing tumour growth.
- Necessary for the protection of myelin sheath in nerve fibres.

Deficiency

- Night blindness - Inability to see in dim light.
- Keratinization - drying, scaling and roughening of skin, eyes and genito-urinary, respiratory and alimentary tract.
- Xerophthalmia - Dryness of the eye causing blindness.
- Faulty bone and teeth growth.
- Decreased resistance to infection and impaired wound healing.
- In breeding animals deficiency leads to abortion, short gestation, retained placenta or production of dead, weak or blind calves.
- In chicks: deficiency causes bone deformities and ruffled feathers, lower resistance to disease and decreased egg production.

Requirement for most of the animals depends upon age, sex and rate of growth and reproduction. It is in the range of 100 to 200 I.U/Kg feed. One I.U. equal to 0.3 μ g of retinol.

Dietary sources

- *Animal sources:* Fish liver oils, Liver of animal – beef, pork and chicken etc., Whole milk, butter, cheese, eggs.
- *Plant sources:* Green leafy vegetables, carrots, mango, pumpkin and papaya.

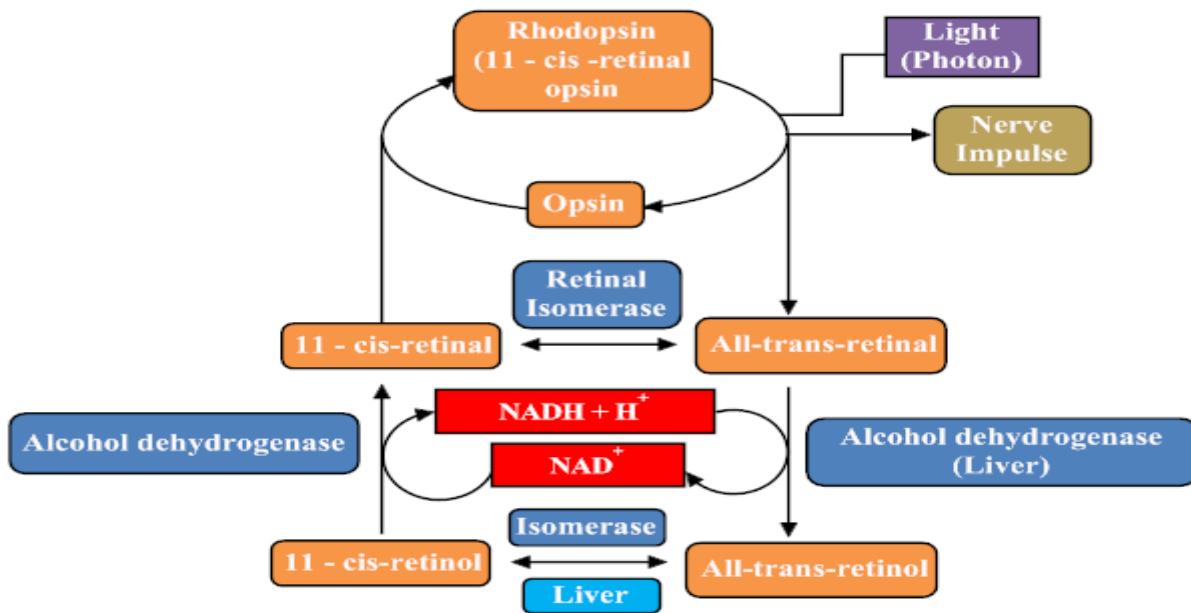
Toxicity

- Vitamin A is toxic, if consumed in excessive amounts leading to bone fragility, vomiting, weakness, loss of appetite, hair loss, diarrhea enlargement of liver and spleen, and skin rashes.

VITAMIN A AND WALD'S VISUAL CYCLE

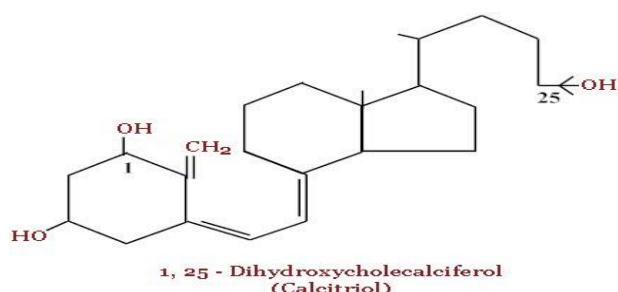
Visual cycle

- Rhodopsin occurs in the rod cells of the retina, which is responsible for vision in poor light.
- *Cis*–retinal, an isomer of all – trans retinal is specifically bound to the visual protein, opsin to form rhodopsin.
- When rhodopsin is exposed to light, it dissociates to form all – trans – retinal and opsin. This reaction is accompanied by a conformational change that induces a calcium channel in the membrane of rod cells.
- The rapid influx of Ca^{2+} ion triggers the nerve impulses allowing light to be perceived by the brain.



VITAMIN D

Chemistry



- The term “Vitamin D” refers to two active forms – Vitamin D₂ and Vitamin D₃.
- Vitamin D₂ (ergocalciferol) is formed from the irradiation of ergosterol (plants and yeast) by UV rays of sunlight.
- Vitamin D₃ (cholecalciferol) is formed in animals by the action of sunlight on 7- dehydro-cholesterol in the skin of the animals.
- Cholesterol and ergosterol are polyisoprenoid compounds. Thus, vitamin D is also made of isoprenoid units.
- Vitamin D₂ and D₃ differ only in the structure of the side chain at the C₁₇ of the steroid nucleus. Ergosterol has an additional double bond between the carbon 22 and 23 and a methyl group at carbon number 24.
- Vitamin D₂ and D₃ are not biologically active. They must be further hydroxylated at carbon 1 and 25 positions, by specific mixed- function oxidases.

- This hydroxylation occurs in two stages. In liver, Vitamin D₃ is first hydroxylated on the 25th position, which is then transported to kidney via blood, where it is hydroxylated on position 1 to form 1, 25- dihydroxycholecalciferol. 25-hydroxy D₃ is the major form of Vitamin D in the circulation and also major storage form in the liver.
- 1, 25 – dihydroxycholecalciferol is the biologically active form of vitamin D₃, which is also known as “calcitriol”.

Metabolic role

- “Calcitriol”, the active form of vitamin-D, functions as hormones, regulating the plasma calcium and phosphate levels. Further,
 - It promotes the absorption of calcium and phosphorus in the intestine.
 - It helps in the mineralization of bone and teeth.
 - It aids in the formation of bone matrix.

Deficiency

- Vitamin D deficiency leads to lowered absorption of calcium, lowered serum calcium levels and reduced bone calcification.
 - Rickets is the deficiency disease, seen in infants, which is characterized by soft, pliable bones.
 - Osteomalacia (adult rickets): The joints are swollen and enlarged.
 - In cows deficiency causes milk fever.
 - In chicks: Thinning of eggshell, slow growth and decreased egg production.

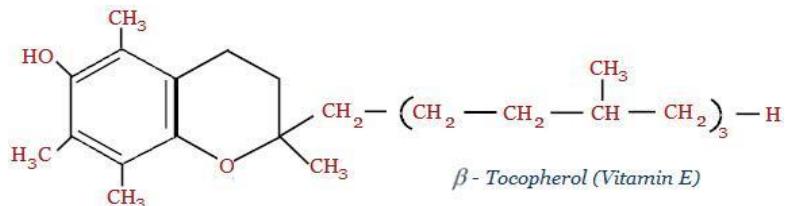
Requirement: For most animals 5 µg / 1000Kg of diet is required.

Source: Fish, liver oils, egg yolk, butter and milk

VITAMIN E

- Vitamin E is the name given to a family of compounds called “tocopherols”.

Chemistry



- The tocopherols are derivatives of 6-hydroxy chromane (tocol) ring with isoprenoid (3 units) side chain.

- There are eight tocopherols known and they are named as alpha -, beta -, gamma - and delta - tocopherols, depending upon the presence of methyl groups in the tocol ring.
- For example, alpha -tocopherol contains methyl groups at position number 5,7 and 8 of the ring. beta - tocopherol has methyl group at 5 & 8 position. gamma- has methyl group at 7 and 8 position and delta - has methyl group at 8 position.
- Alpha -tocopherol is the most active form of all these structure.

Biological Functions

- The principal function of vitamin E is to act as an antioxidant.Prevents the oxidation of vitamin A in the small intestine.
- Protects vitamin C and unsaturated fatty acid from oxidation.
- Prevents the hemolysis of red blood cells.
- Prevents the formation of free radicals, thereby, protects cell membranes.
- Assists in cellular respiration.
- It seems to be involved in heme synthesis.
- Vitamin E aids in the normal phosphorylation of creatine and ATP.
- High intake of vitamin E protects against the development of heart diseases.

Deficiency

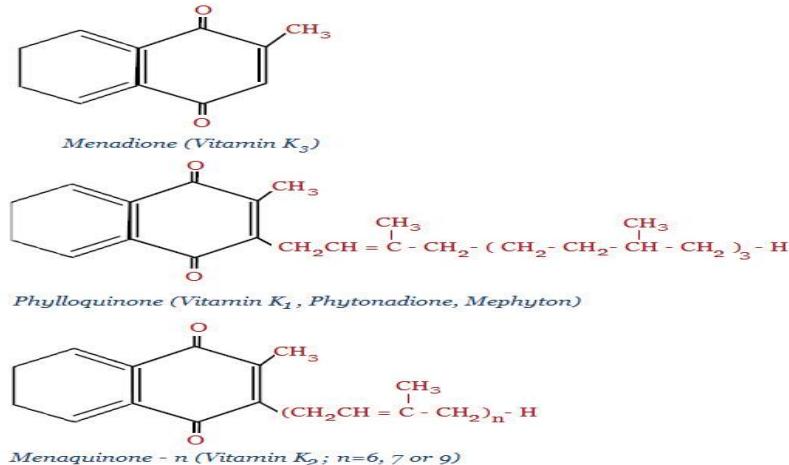
- Increases hemolysis of red blood cells and anemia due to decreased production of hemoglobin.
- Necrosis of the liver
- Reproductive failure in male as well as in female. Hence vitamin E is also known as antisterility factor.
- Muscular dystrophy, which leads to difficulty in standing, tremoring and staggering gait (white muscle disease) in cattle and stiff lamb disease in lambs.
- Impaired lipid metabolism.
- In chicks:
 - Exudative diathesis (leakage of plasma into the tissues)
 - Encephalomalacia (damage to brain, edema and hemorrhage).
- Symptoms are ataxia, muscular in-coordination, head retraction and cycling with legs.

Sources: Vegetable oils and fats, Nuts and oil seeds, Liver, butter and milk.

VITAMIN K

- Vitamin K is the only fat soluble vitamin with a specific co-enzyme function.

Chemistry



- All the compounds of this group are naphthoquinone derivatives with isoprenoid side chains of varying length.
- Vitamin K₁: Phylloquinone, is found in plants.
- Vitamin K₂: Menaquinone is synthesized in the intestinal tract of animals.
- Vitamin K₃: Menadione is the synthetic form of vitamin K and the parent compound of the Vit K series.
- Dicoumarol (Sweet Clover poisoning or bleeding disease), a naturally occurring anti-coagulant, present in spoiled sweet clover, inhibits the synthesis of Vitamin-K.
- Synthetic analog, warfarin, also inhibits competitively the formation of prothrombin.

Functions

- Vitamin K is necessary for the formation of gamma-carboxyglutamate in the prothrombin, which binds with the Ca²⁺.
- Vitamin K is also essential for the synthesis of several other Ca²⁺ binding proteins like osteocalcin, a non-collagen protein found in bone, which contains γ-carboxyglutamate residues.
- It is involved in the maintenance of some blood-clotting factors like II, VII, IX and X. Hence, it is called “anti-hemorrhagic vitamin”.
- Vitamin K is structurally related to ubiquinones, the components of electron transport chain.

Deficiency

- Symptoms have not been reported in ruminants, horse and pigs under normal conditions. The microflora of the GI tract supplies sufficient amounts to the animal.
- In general deficiency leads to a lowering of prothrombin concentration and increased clotting time of blood.

- Hemorrhagic disease of the newborn (a bleeding disorder)
- In chicks: Decreased blood clotting time, hemorrhage in muscle and anemia and mortality

Requirement: 500-1000 µg/Kg diet.

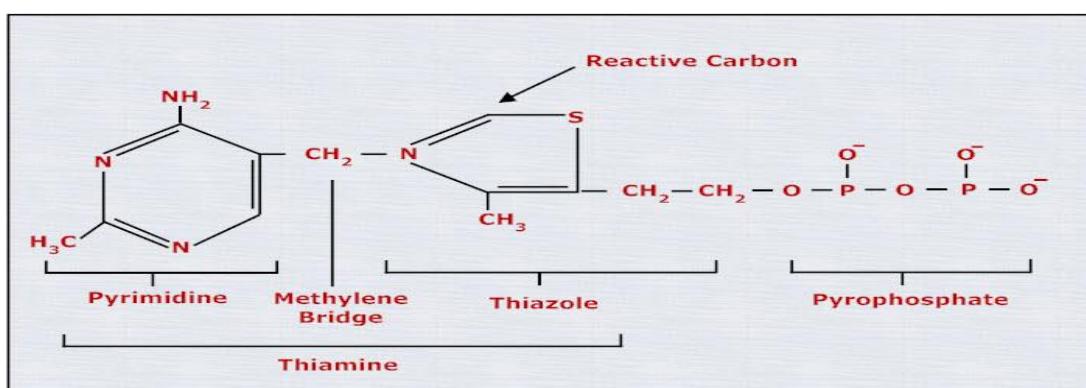
Sources: Green vegetables such as spinach, cabbage, cauliflower, tomato and soya beans, egg yolk and liver.

WATER SOLUBLE VITAMINS

VITAMIN B1 (THIAMINE)

- Also known as “aneurin” or “anti- beri-beri factor”.

Chemistry



- Thiamine contains 2 ring systems, a pyrimidine and a thiazole ring joined by a methylene bridge.
- Thiamine pyrophosphate (TPP) is the coenzyme form of thiamin.
- Thiamine is heat and alkali labile. The level is reduced during various steps in food processing. Thiamine can be destroyed by an enzyme thiaminase, which is present in the flesh of some fish.
- An ATP dependant thiamine diphospho transferase, present in brain and liver is responsible for the conversion of thiamine to TPP.

Functions

- TPP is a co-factor in the following reactions
- Pyruvate dehydrogenase complex catalyzes the conversion of pyruvate to acetyl CoA linking the glycolytic cycle with TCA.
- α- ketoglutarate dehydrogenase catalyses the conversion of a α- ketoglutarate to succinyl-CoA in citric acid cycle.

- Transketolase: Involves in the transfer of ketose phosphate in HMP shunt for the oxidation of glucose to produce ribose and NADPH.
- In the oxidative decarboxylation of branched chain amino acids (leucine, isoleucine and valine).
- Release of energy from fat and carbohydrates.
- Promotes better appetite and functioning of the digestive tract and also in cardiac Functions.
- TPP is involved in the flux of sodium ion across neural cell membrane.
- In chicks: nervousness, head retraction, inability to stand, poor growth rate and decreased egg production.

Requirement: Animals should receive 4 to 10 mg of thiamin/kg dry food.

Deficiency

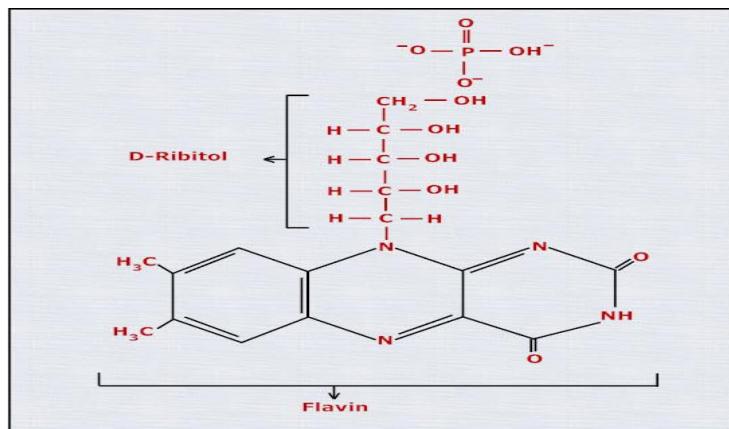
- Thiamin deficiency causes the clinical neurological disorder known as beri – beri. (beri-beri = sheep, as the thiamin deficient patients walk with their knees shaking and the legs raised up).
- Dry beri beri is characterized by weight loss, muscle weakness and nervous disorders.
- In Wet beri beri, edema is present due to cardiac failure.
- Presence of tannins reduces the availability of thiamin.

Sources: Dried yeast, rice polishing, wheat germ, legumes, oil seeds and nuts. Liver, meat and fish.

VITAMIN B2 (RIBOFLAVIN)

Chemistry

- Riboflavin consists of a dimethyl heterocyclic isoalloxazine ring attached to a sugar alcohol, ribitol.
- It is an orange- yellow coloured, fluorescent pigment that is heat stable, but decomposes in the presence of visible light. The intense yellow color is due to its complex “isoalloxazine ring system”.
- The 2 biologically active co-enzyme forms are Flavin Mono Nucleotide (FMN) and Flavin Adenine Dinucleotide (FAD).



Functions

- FMN and FAD serve as prosthetic groups for oxido-reductase enzymes. These enzymes are known as flavoproteins.
- *NADH dehydrogenase*: A component of respiratory chain in mitochondria
- *Succinate dehydrogenase*: Succinate to fumarate in citric acid cycle
- *Acyl-CoA dehydrogenase*: Acyl-CoA to α , β -unsaturated acyl-CoA in fatty acid oxidation.
- *Xanthine oxidase*: Hypoxanthine to xanthine and xanthine to uric acid in purine catabolism.
- L-amino acid oxidase catalyzes the oxidation of L-amino acids.
- Release of energy from fat, carbohydrates and proteins.
- Essential for the healthy skin and growth.
- Normal functioning of the eye.

Deficiency

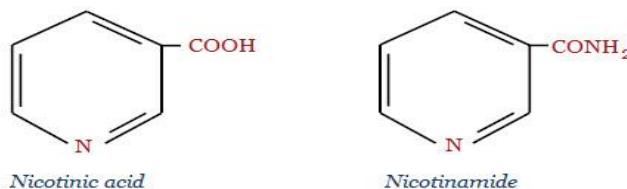
- *Cheilosis*: A cracked or broken lesions on the lips and corners of the mouth (stomatitis).
- *Glossitis* (inflammation of the tongue).
- *Seborrhea* (excessive secretion of sebum in sebaceous glands, which is more greasy and rancid in odour).
- *Photophobic* (hating light).
- In pigs growth is affected. In sows deficiency affects oestrous activity.
- Curled toe paralysis in chickens. Deficiency also causes reduced hatchability and embryonic abnormalities.

Sources: Liver, kidney, milk, cheese, fish, eggs, yeast and green leafy vegetables.

VITAMIN B3 (NIACIN)

- Previously known as “Nicotinic acid” and its amide derivative nicotinamide.
- Tryptophan present in the dietary protein could be converted into niacin in the body. (60 mg tryptophan = 1 mg of niacin).
- In cat tryptophan cannot produce niacin.
- Excess dietary leucine can bring about niacin deficiency by inhibiting quinolinate phosphoribosyl transferase, a key enzyme in the conversion of tryptophan to NAD.
- Vit. B₆ deficiency can also potentiate the deficiency of niacin.

Chemistry



- Nicotinic acid is a monocarboxylic acid derivative of pyridine (pyridine-3-carboxylic acid)
- The biologically active coenzyme forms are, nicotinamide adenine dinucleotide (NAD⁺) and it's phosphorylated derivative (NADP⁺).
- They are involved in several oxido-reduction reactions.

Functions

NAD as coenzyme

- Glyceraldehyde 3-phosphate dehydrogenase: Glyceraldehyde to 1,3 bisphosphoglycerate (glycolytic cycle).
- Lactate dehydrogenase: Pyruvate to lactate or lactate to pyruvate (glycolytic cycle).
- Pyruvate dehydrogenase complex: Pyruvate to acetyl-CoA (mitochondria).
- Isocitrate dehydrogenase: Isocitrate to α-ketoglutarate (citric acid cycle).
- Malate dehydrogenase: Malate to oxaloacetate (citric acid cycle).
- β-hydroxyacyl-CoA dehydrogenase: β-hydroxyacyl-CoA to β-ketoacyl-CoA (fatty acid oxidation).
- Glutamate dehydrogenase: Glutamate to α- ketoglutarate (amino acid Metabolism). NADP is also used as a coenzyme.
- Release energy from carbohydrates, proteins and fats.
- Synthesis of proteins, nucleic acids and fatty acids.
- 10 Essential for healthy skin and normal functioning of GI tract and nervous system.

NADP as coenzyme

- Glucose 6-phosphate dehydrogenase: Glucose 6-phosphate to 6- Phospho- gluconolactone (HMP shunt).

- HMG CoA reductase: HMG CoA to mevalonate (cholesterol synthesis).

Deficiency

- Deficiency causes “pellagra”. The disease is characterized by three D’s.
- *Dermatitis* : Inflammation of the skin.
- *Diarrhea* : Due to inflammation of the gastro- intestinal tract.
- *Dementia* : Mental confusion. If not treated, the ultimate outcome is death.
- *In Dogs*: Black tongue, which is characterized by reddening of the mucosa of lips and mouth, which leads to necrosis of the mucosa accompanied by ropy saliva, a fetid odour and diarrhea.
- *In chicks*: Black tongue, leg bone abnormalities.

Requirement: 10 – 25 mg/Kg diet, which depends on species.

Therapeutic Use:

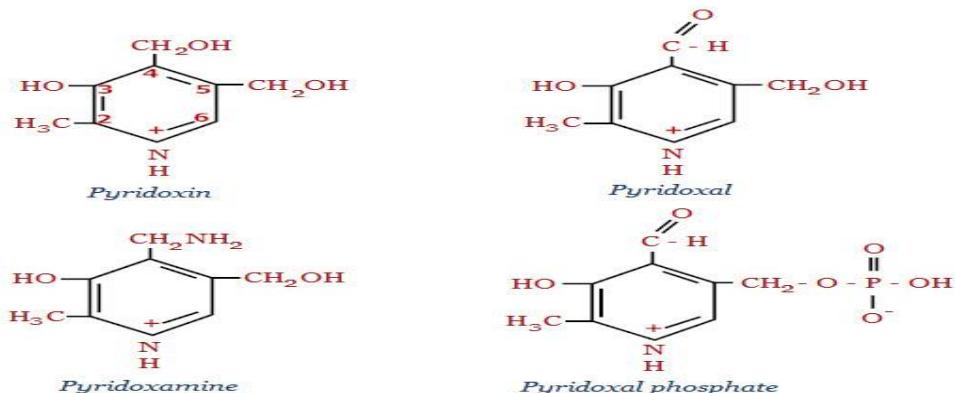
- Nicotinic acid has been used therapeutically for lowering plasma cholesterol.
- This is due to inhibition of the flux of free fatty acid from adipose tissue, which leads to less formation of cholesterol bearing lipoproteins - VLDL & LDL.

Source : Liver, kidney, eggs meat, milk, fish, yeast, and peanut. Niacin can be obtained by the hydrolysis of NAD and NADP. Corn is deficient of niacin and tryptophan.

VITAMIN B₆ (PYRIDOXINE)

- Vitamin B₆ consists of 3 closely related compounds known as pyridoxine, pyridoxal and pyridoxamine and their corresponding phosphates.

Chemistry



- All are derivatives of pyridines.
- The active co- enzyme form of Vitamin B₆ is pyridoxal phosphate

Functions

- Pyridoxal phosphate serves as coenzyme in numerous enzymes that are involved in amino acid metabolism.

Transamination reactions

- Alanine to pyruvate
- Oxaloacetate to aspartate
- *In the decarboxylation reactions*
 - Glutamate to γ - aminobutyric acid
 - Tryptophan to serotonin
 - Tyrosine to norepinephrine
 - Serine to glycine.
- *In the synthesis of heme*
 - Glycine and succinyl-CoA are condensed to δ - aminolevulinic acid
- *In glycogenolysis*
 - Glycogen phosphorylase requires this coenzyme for effective function
- *Production of antibodies.*
- *Conversion of linoleic acid to arachidonic acid.*

Deficiency

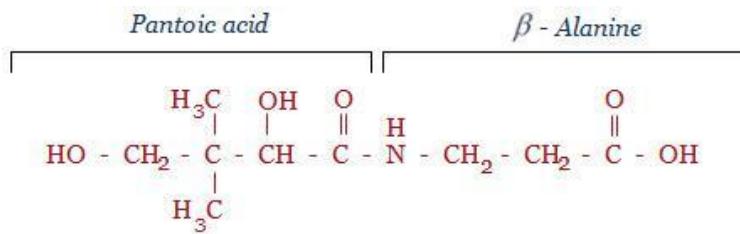
- Deficiency is rare. Deficiency of this vitamin leads to
 - Neurological disorder due to reduced synthesis of biogenic amines from amino acids.
 - Anemia due to reduced heme synthesis.
 - Some animals like cat excrete oxalic acid (oxalouric).
 - Isoniazid, a drug used in the treatment of tuberculosis can cause B_6 deficiency.
 - In chicks: Nervous disorder, leg bone abnormalities, ataxia.

Requirements: Normally 2 to 6 mg /Kg diet is required.

Sources : Meat, cereals, soyabeans, peanuts, yeast, banana, fish, poultry, wheat bran and wheat germ.

PANTOTHENIC ACID

Chemistry



- Pantothenic acid is formed by the combination of pantoic acid and β - alanine linked by a peptide bond.
- It is then phosphorylated to form phosphopantothenate. Addition of cysteine followed by the removal of its carboxyl group produces phosphopantetheine. It is present at the active site of acyl carrier protein (ACP) a component of fatty acid synthesis complex and CoA.

Functions

- It is required for the metabolism of fat, protein and carbohydrate via the citric acid cycle. More than 70 enzymes utilize CoA or its derivatives for their activity.
- In CoA (A= acetylation), SH is the reactive site. Thus, Coenzyme-A is usually referred as “CoA-SH”.
- It is used in the synthesis of fatty acids, cholesterol and other sterols. Co-A forms thioester bonds with the free fatty acids resulting in the formation of acyl-Co A.
- It is a carrier of activated acyl groups. Eg: acetyl- Co A.
- Acetyl-CoA is a central molecule for a wide variety of biochemical reactions.
- Co-enzyme A is useful for group transfer reactions.
- Eg: It is involved in acetylcholine synthesis
- Acetyl-CoA + choline \rightarrow Acetylcholine + Co A
- Acetyl –CoA + oxaloacetate \rightarrow Citrate + Co A
- It is used in the synthesis of porphyrin ring, which is part of the hemoglobin.

Requirement is 10 to 20 mg/kg diet.

Deficiency

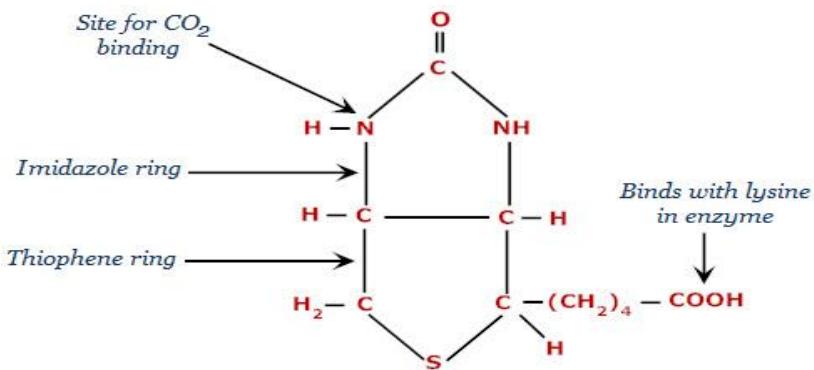
- Pantothenic acid deficiency is not common. Induced deficiency causes headaches, vomiting, indigestion, depression and lack of co-ordination.
- In pigs deficiency causes slow growth, diarrhea, loss of hair and a characteristic goose-stepping gait.
- In chicks: Ataxia and leg bone abnormalities

Sources: Liver, kidney, fish, whole grains, milk, fruits and vegetables.

BIOTIN

Chemistry

- Biotin is an imidazole derivative, which is fused with a thiophene ring containing valeric acid as a side chain.



Functions

- Biotin functions as coenzymes for cellular carboxylation reactions.
- Pyruvate carboxylase: Pyruvate to oxaloacetate (in gluconeogenesis and for replenishment of the citric acid cycle)
- Acetyl-CoA carboxylase: Acetyl-CoA is carboxylated to malonyl-CoA (fatty acid synthesis)
- Propionyl-CoA carboxylase: Conversion of propionyl-CoA to methyl malonyl-CoA (gluconeogenesis)
- Synthesis of purines that are important constituents of DNA and RNA.

Deficiency

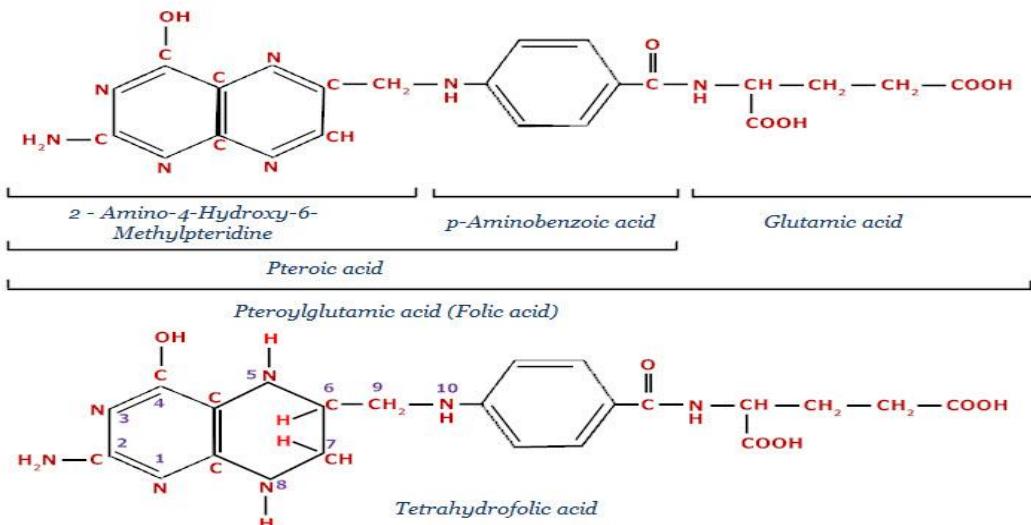
- Since biotin is synthesized by the intestinal flora, biotin deficiency has not been observed normally.
- Raw egg contains a protein called "Avidin", which binds with biotin very tightly, which makes biotin deficiency.
- Biotin deficiency has been induced experimentally by eliminating biotin intake and either sterilizing the intestinal tract or feeding raw egg white.
- Induced biotin deficiency will lead to muscle pain, dermatitis, glossitis and vomiting.
- Increase in blood cholesterol level.
- Depression of gluconeogenic process and fat metabolism.
- Deficiency causes a condition called egg white injury in fur bearing animals and pigs.
- In chicks: Due to deficiency of pyruvate carboxylase, pyruvate is not converted to oxaloacetate for glucose synthesis, which causes fatty liver kidney syndrome (FLKS). Leg bone abnormalities and dermatitis are also seen.

Requirement is low, which is in the range of microgram/Kg diet.

Sources: Liver, kidney, egg yolk, yeast, milk and peanuts.

FOLIC ACID

Chemistry



- It is chemically known as “*Pteroylglutamic acid*”, which contains three major components – glutamic acid, p-aminobenzoic acid (PABA) and a base pteridine.

Functions

- Folic acid is converted to tetrahydrofolate, which is the coenzyme form of folic acid. Folic acid carries one carbon unit, which may be in the form of methyl (-CH₃), methylene (-CH₂-), methenyl (-CH=), formyl (-CHO), and formimino (-CH=NH) groups that are transferred from one molecule to another in the cell. The one carbon group is covalently bound to N-5 or N-10 or to both in a ring form.

It participates in the following reactions

- In the conversion of homocysteine to methionine.
- In the metabolism of glycine, serine and choline
- In the synthesis of thymine required for DNA synthesis.
- In the synthesis of purine base: the carbon 2 and 8 are obtained from THF.
- It is necessary for the formation of red blood cells.
- Dihydrofolate reductase, an enzyme, which converts dihydrofolate to tetrahydrofolate is completely inhibited by methotrexate, a folic acid analogue.
- Sulfanilamide and its derivatives are structural analogues of p-aminobenzoic acid (PABA). These drugs completely inhibit the synthesis of folic acid and thereby decreases the synthesis of nucleotide necessary for the formation of DNA and RNA. Sulfa drugs do not affect human DNA or RNA formation because mammalian cells cannot synthesize folic acid.

Deficiency

- As synthesized by the microflora, deficiency is less common in ruminants.
- Slows growth interferes with cell regeneration.
- *Macrocytic anemia*: Red blood cells are large and too few and have less hemoglobin than normal.
- *Megaloblastic anemia*: Large nucleated cells with odd shape. Diminished synthesis of purine and pyrimidines leads to an incapability of cells to make DNA and RNA and to divide.
- In chicks: leg bone abnormalities, Poor growth.

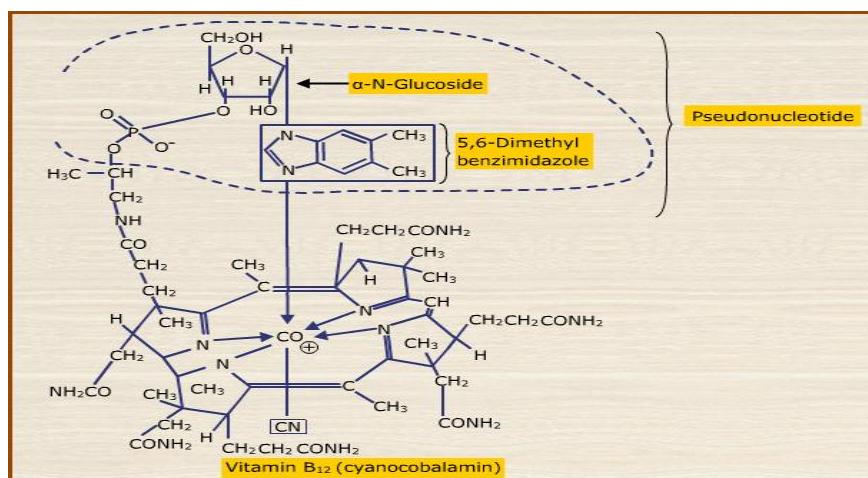
Requirement ranges from 1 to 10mg/kg diet.

Sources: Liver, kidney, yeast, fish, green leafy vegetables, sweet potatoes and pumpkin.

VITAMIN B₁₂

Chemistry

- Vitamin B₁₂ has a complex corrin ring structure, which is similar to porphyrin ring found in heme.
- Cobalt is held in the center of the corrin ring by four co-ordination bonds from the nitrogens of the pyrrole groups.
- The remaining co-ordination bonds of the Co are with the nitrogen of 5, 6-dimethyl benzimidazole, and with CN in commercial preparation of the vitamin in the form of cyanocobalamin.
- The active B₁₂ coenzymes are:
 - Methylcobalamin and
 - Deoxyadenosylcobalamin.



Functions

- Vitamin B₁₂ is involved in the reactions in two forms
 - *Methylcobalamin* : Vit.B₁₂ is needed for the transfer of methyl group from tetrahydrofolate to homocysteine to form methionine. Deficiency leads to an accumulation of N₅-methyl-tetrahydrofolate called folate trap.
 - *5'-deoxyadenosylcobalamin* : In the conversion of propionic acid to glucose, the methyl group of L-methylmalonyl-CoA is rearranged to form succinyl-CoA in a reaction catalyzed by methylmalonylCoA isomerase. This reaction requires Vitamin B₁₂. In the absence of Vit.B₁₂ methylmalonic acid accumulates in blood and is excreted in urine.
- It aids in the formation of red blood cells.
- Normal functions of all body cells especially gastrointestinal tract and nervous system.
- It helps in the bone marrow formation.
- It is used in the prevention of pernicious anemia.
- Needed for the synthesis of DNA and RNA.

Deficiency

- Adult animals are less affected by deficiency than young ones. In chicks, growth and hatchability are affected.
- Due to the lack of an intrinsic factor (a glycoprotein secreted by the parietal cells of stomach mucosa that promotes B₁₂ absorption), pernicious anemia develops, which is characterized by the deficiency in red blood cells, hemoglobin concentration and severe impairment of CNS. The neurological disorders seen in vitamin-B12 deficiency are due to progressive demyelination of nervous tissues. Two pathways are proposed for the condition.
 - Methylmalonyl-CoA is a competitive inhibitor of malonyl-CoA in fatty acid synthesis. Since, myelin sheath is continually turning over, any severe inhibition of fatty acid synthesis will lead to degeneration.
 - In the fatty acid synthesis, methylmalonyl-CoA can substitute for malonyl-CoA, leading to branched chain fatty acids, which might disrupt normal membrane structure.
- Glossitis, anorexia, weakness, weight loss, mental and nervous symptoms.
- Deficiency also leads to megaloblastic anemia. In vitamin-B₁₂ deficiency, the N₅ – methyl form of tetrahydrofolate is not efficiently used. Thus, N₅-methyl form accumulates, and other forms of tetrahydrofolate are not formed, resulting in the decreased synthesis of purine and pyrimidine, which results in megaloblastic anemia.

Requirement is in the range of 2-15 µg /Kg diet.

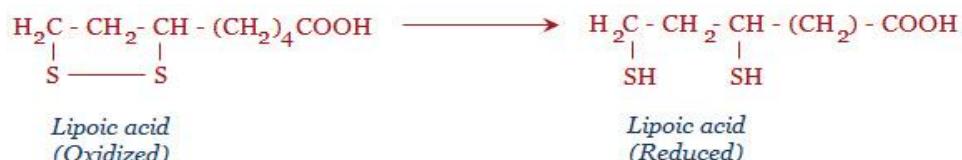
Sources: Animal products like organ meat, eggs shrimp, chicken and pork.

LIPOIC ACID

- It is a growth factor for certain microorganisms and protozoa. Thus, it can be termed as vitamins. Animals can synthesize the amount required.

Chemistry

- Lipoic acid (6,8-dithio octanoic acid) is a sulfur containing fatty acid.
- Lipoic acid exists in 2 forms.
 - A closed ring disulfide (oxidized) form and
 - An open chain reduced form.
- Usually, lipoic acid does not exist freely in nature. It is always covalently attached to lysine of the enzyme protein by an amide linkage.



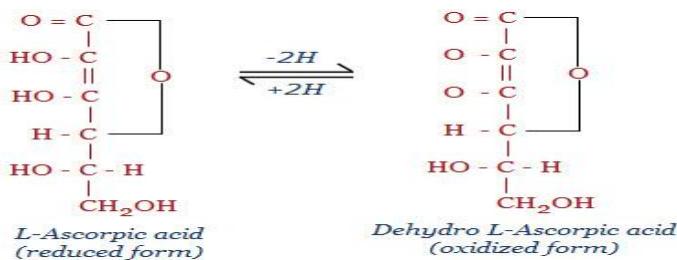
Functions

- It is a cofactor for multienzyme complexes, like, pyruvate dehydrogenase and α -ketoglutarate dehydrogenase reactions, which are involved in carbohydrate metabolism. Lipoic acid functions to couple acyl group transfer and electron transfer during oxidation and decarboxylation of α -keto acids.

Sources: Liver and yeast are the richest sources.

VITAMIN C

Chemistry



- Ascorbic acid is a six-carbon, organic compound, resembles the structure of glucose.
- It occurs in 2 active forms, the reduced form (ascorbic acid) and the oxidized form (dehydro-ascorbic acid).
- Most mammals synthesize ascorbic acid from glucose.
- The enzyme gulonolactone oxidase, which is necessary for the synthesis is deficient in man, monkey and guinea pigs. Hence, these mammals cannot synthesize vitamin C.

- Farm animals can synthesize this vitamin, deficiency symptoms normally do not arise.

Functions

- It is essential for the synthesis of collagen through the hydroxylation of proline and lysine, which aids in the healing of wounds.
- It enhances absorption of iron from the small intestine and also to keep the iron in the reduced state.
- It aids in the conversion of cholesterol to bile acids.
- In the synthesis of epinephrine from tyrosine.
- Converts the amino acid tryptophan to serotonin.
- In the degradation of tyrosine.
- Aids in the synthesis of steroids by the adrenals.
- It acts as a water- soluble antioxidant.
- Aids in the synthesis of mucopolysaccharides, which helps the formation of bone and teeth.
- Improves resistance to infection.
- Helps to maintain elasticity of blood vessels and capillaries.

Deficiency

- Ascorbic acid deficiency causes Scurvy, which is characterized by hemorrhage, anemia, loose teeth, bleeding of gums, swollen joints, delayed wound healing and susceptibility to infections.

Sources : Citrus fruits like orange, lemon and grapefruits, Green leafy vegetables, tomatoes, apple guava and pineapple. Animal sources are very poor in vitamin C.

INTEGRATION OF METABOLISM

- The purpose of metabolism is to oxidize the food to provide energy in the form of ATP.
- Some molecules obtained during metabolism are also converted to new cellular materials and essential components.
- Another important function is the processing of waste products to facilitate their excretion.
- Animals take food at periodic intervals, they do not eat continuously. After a meal, blood is loaded with glucose, triacylglycerol and amino acids, which are absorbed from the intestine. From the blood these nutrients are taken into tissues so that blood levels are quickly returned to normal level. The tissues store the excess food and use these stores when the animals are fasted. There are regulatory mechanisms, which direct compounds through metabolic pathways involved in storage and utilize them as fuel when required.
- Hormones (like insulin, glucagon and catecholamines), concentration of available nutrients and the energy needs of the body control the mechanisms.

Carbohydrate

- Glucose is present as glucose 6-phosphate in liver. As liver contains glucose 6-phosphatase, free glucose is released from liver to blood from glucose 6-phosphate.
- Glucose 6-phosphate is oxidized in glycolytic cycle to produce energy.
- Glucose 6-phosphate is converted to acetyl-CoA, which is used to synthesize fat and cholesterol.
- Excess glucose 6-phosphate is stored as glycogen.
- Glucose 6-phosphate is shunted to HMP pathway to form ribose-5-phosphate and NADPH needed to nucleic acid and fatty acid biosynthesis respectively.

Fat

- Fatty acids are oxidized to acetyl-CoA, which is used to produce energy.
- Acetyl-CoA is used to form ketone bodies, which are fuels in extra-hepatic tissues.
- Unsaturated fatty acids are used to synthesize autacoids / eicosanoids (PG, TX and LT).
- Cholesterol and steroid hormones are also synthesized from acetyl-CoA.
- Excess fatty acids are stored as triacylglycerol in adipose tissues.

Amino acids

- Amino acids are used to synthesize enzymes, transport proteins, other important body proteins including plasma proteins.
- Several biologically active peptides such as neuropeptides are produced.
- Amino acids are precursors of several hormones.
- Amino acids can be catabolised to acetyl-CoA for oxidation to produce energy or intermediates of TCA cycle, which may also be oxidized to provide energy or used to synthesize glucose via gluconeogenesis.

- Acetyl-CoA formed is also used to synthesize fatty acid and stored as fat.
- The ammonia released during deamination of the amino acid is taken to liver for conversion into Urea and is excreted by kidneys

REACTIONS IN LIVER - UNDER WELL FED STATE

Liver on carbohydrate metabolism

- Liver plays a central role in maintaining the blood glucose level. When blood glucose is high such as after a meal, the liver takes up and stores glucose as glycogen. When blood glucose is low, liver breaks down the glycogen and releases the glucose into blood circulation. Glucokinase present only in the liver is involved in taking more glucose from the blood. This enzyme has got high Km for glucose; it is most active when the glucose level is high. High insulin / glucagons ratio activates glycogen synthetase which promotes glycogen synthesis.

Liver on lipid metabolism

- Excess dietary carbohydrates are converted to triacylglycerol in the liver. The TG is transported from the liver as VLDL. The lipoprotein lipase, which is needed for the hydrolysis of TG from VLDL and chylomicron is induced by insulin (excess glucose in blood stimulates insulin production). The fatty acids are taken up by adipose tissue and stored as TG in adipose tissues.

Liver on protein metabolism

- It is the important site for amino acid metabolism. After a protein rich meal, the liver uses amino acids for the synthesis of proteins. The surplus amino acids are deaminated; the carbon skeleton is converted to pyruvate, other TCA cycle intermediates and acetyl-CoA . Pyruvate and TCA cycle metabolites can be converted to glucose through gluconeogenesis that can be released into the blood. These metabolites can also be oxidized to get energy. The acetyl CoA can be oxidized to provide energy or used for fatty acid biosynthesis.
- The liver contains urea cycle that converts toxic ammonia to non toxic urea.
- The carbon skeleton and nitrogen of amino acids are also used for the synthesis of nitrogen containing compounds such as heme, purines and pyrimidines.

REACTIONS IN MUSCLE - UNDER WELL FED STATE

Muscle on carbohydrate metabolism

- Muscle takes up glucose from the blood and stores it as glycogen. In contrast to liver, muscles do not release free glucose into the blood. Increased insulin level increases muscle glycogen synthetase activity. Muscle prefers fatty acids and ketone bodies for oxidation to provide energy. The lactate formed during muscle contraction is taken up by liver to produce glucose, which are then released into blood and taken up by muscle to meet the energy demand.

Muscle on lipid metabolism

- By the action of lipoprotein lipase on VLDL and chylomicron, free fatty acids are liberated and are used secondarily as fuel. When glucose is available it is used first for producing energy.

Muscle on protein metabolism

- In muscle the insulin released after a protein meal allows the uptake of amino acids and stimulates protein synthesis. The branched chain amino acids are used for muscle protein synthesis and are also metabolized to obtain energy.

REACTIONS IN ADIPOSE TISSUE - UNDER WELL FED STATE

Adipose tissue on carbohydrate metabolism

- Glucose can be taken up by adipose tissue for fat synthesis. High insulin/glucagon ratio activates triacylglycerol synthesis in adipose tissue. Increased glucose uptake provides glycerol 3-phosphate for esterification of fatty acids. Increased activity of pyruvate dehydrogenase complex provides acetyl-CoA for fatty acid synthesis. Adipose tissue metabolizes glucose through HMP shunt producing NADPH, which is needed for fatty acid synthesis.

Adipose tissue on fat metabolism

- Fat is stored in the form of triacylglycerol, which is the major storage form in adipose tissue. During high carbohydrate diet, the transport of glucose into the adipocyte is stimulated by insulin. Some fatty acids that are incorporated into triacylglycerol are synthesized within the adipocyte from glucose.

REACTIONS IN BRAIN AND ERYTHROCYTES - UNDER WELL FED STATE

Brain

- Glucose is the main fuel for brain tissue. Brain can not use fatty acid as fuel because they are bound to albumin in plasma.
- Brain contains very small amount of stored glycogen. This tissue needs continuous supply of glucose. Hence, glucose level of blood should be maintained within the normal limit by the liver.
- The amino acids are used for the formation of glutamine and neurotransmitters.

Erythrocytes

- Due to lack of mitochondria red blood cells use only glucose for energy producing lactate.

REACTIONS IN LIVER - UNDER STARVATION

Liver on carbohydrate metabolism

- During fasting the glucose level is lowered and the insulin level is decreased. The pancreas releases glucagon in response to low level of blood glucose (insulin /glucagon ratio is decreased). This change stimulates the liver to break down glycogen and release glucose into blood. Glucagon also inhibits the synthesis of glycogen in the liver and stimulates gluconeogenesis. All of these processes contribute to an increased glucose level in blood. Glycerol released by the degradation of triacylglycerol in adipose tissue, lactate and pyruvate

formed in peripheral tissues, glucogenic amino acids released from the degradation of muscle protein are returned to liver and are then used as substrates for gluconeogenesis.

Liver on lipid metabolism

- When the glucose concentration is low, insulin level drops, the level of glucagon increases, which causes a raise in cellular cAMP level and lipolysis is stimulated. The fatty acids obtained from adipose tissues are oxidized in the liver to produce energy. When the concentration of acetyl-CoA is more than the oxidative capacity of the Krebs cycle, the acetyl-CoA is shunted into ketone body synthesis. Ketone bodies are soluble form of fatty acids. They can be taken up by many organs and tissues, in which the ketone bodies serve as a good source of energy.

Liver on protein metabolism

- Release of amino acids from skeletal muscle is stimulated due to reduced insulin and increased glucocorticoids. Liver takes up the amino acid to produce glucose.

REACTIONS IN MUSCLE - UNDER STARVATION

Muscle on carbohydrate metabolism

- In fasting, reduced blood glucose concentration suppresses the secretion of insulin, which inhibits glucose transport into skeletal muscle through insulin dependent glucose transport proteins. As a result glucose metabolism is reduced.

Muscle on lipid metabolism

- When the blood glucose concentration falls, fatty acids are taken up and oxidized by muscle. Fatty acids are mobilized from adipose tissue. This reduces the demand on blood glucose (it is maintained by glycogenolysis and gluconeogenesis in liver).

Muscle on protein metabolism

- During fasting, degradation of muscle protein, releases amino acids that are used by liver for glucose synthesis.
- Fasting muscle oxidizes branched chain amino acid to produce energy and glutamine.

REACTIONS IN ADIPOSE TISSUE - UNDER STARVATION

Adipose tissue on carbohydrate metabolism

- Due to low level of glucose, insulin production is reduced, which results in the decreased transport of glucose and reduced fatty acid and triacylglycerol synthesis. Glucose level inside the adipocytes determines whether fatty acids are released into the blood (glycerol 3-phosphate needed for esterification is produced from glucose).

Adipose tissue on fat metabolism

- The starvation elevates epinephrine and glucagon levels, which stimulate the activity of hormone sensitive lipase. This hormone hydrolyzes triacylglycerol to free fatty acids, which are taken to various tissues as fuel. The glycerol is taken to liver for the synthesis of glucose. As insulin level is reduced lipoprotein lipase activity is low. Consequently triacylglycerol of lipoprotein is not hydrolyzed to obtain free fatty acids.
- In humans and also in hibernating animals the adipose tissues are called as brown fat, which generates heat rather than ATP production during oxidative phosphorylation.

REACTIONS IN BRAIN - UNDER STARVATION

- When the blood glucose level falls below the critical level, brain functions are affected. Brain adapts its metabolism to use ketone bodies as an energy source. The blood glucose spared by the brain is used by the red blood cells, which can not use ketone bodies for energy because they do not have mitochondria and hence citric acid cycle.
- Brain uses branched chain amino acid for energy. The amino acids also provide nitrogen for the synthesis of neurotransmitters.

INTEGRATION OF METABOLISM - CARBOHYDRATES

Integration of Metabolism				
Carbohydrate metabolism : under well fed state				
Liver	Muscle	Adipose tissue	Brain	RBC
During high glucose level in blood, insulin is produced, which activates glycogen synthase. Liver takes up glucose and converts glucose to glycogen. Glucokinase of liver is involved in taking of glucose from blood and phosphorylating glucose to glucose-6-phosphate. It has got high Km. It is most active when the glucose is high	Increased level of insulin transports more of glucose into muscle cells and is stored as glycogen. The lactate formed during muscle contraction is transported to liver to form glucose. Heart muscle Heart is aerobic and uses glucose, free fatty acids and ketone bodies as fuel. Heart muscle does not store lipids and glycogen in large amounts.	Increased level of insulin transports more of glucose into adipose tissue and is converted to glycerol-3-phosphate for the esterification of fatty acids to form triacylglycerol. This tissue also metabolizes glucose through HMP shunt producing NADPH, which is needed for fatty acid synthesis.	Glucose is the main fuel for brain tissue. Brain cannot use fatty acid as fuel because they are bound to albumin in plasma and hence cannot cross the 'BBB'. Brain contains very small amount of stored glycogen. This tissue needs continuous supply of glucose. Hence, glucose level of blood should be maintained within the normal limit by the liver.	Due to lack of mitochondria red blood cells use only glucose for energy production producing lactate.

Carbohydrate metabolism : under starvation				
<p>During fasting the glucose level is lowered and the insulin level is decreased. The pancreas releases glucagon in response to low level of blood glucose (insulin/glucagon ratio is decreased).</p> <p>This change stimulates the liver to break down glycogen and release glucose into blood. Glucagon also inhibits the synthesis of glycogen in the liver and stimulates gluconeogenesis. All of these processes contribute to an increased glucose level in blood.</p> <p>Glycerol released by the degradation of triacylglycerol in adipose tissue, lactate and pyruvate formed in peripheral tissues, glucogenic amino acids released from the degradation of muscle proteins are returned to liver and are then used as substrates for gluconeogenesis.</p>	<p>In fasting, reduced blood glucose concentration suppresses the secretion of insulin, which inhibits glucose transport into skeletal muscle through insulin dependant glucose transport proteins. As a result glucose metabolism is reduced.</p>	<p>Due to low level of glucose, insulin production is reduced, which results in the decreased transport of glucose and reduced fatty acid and triacylglycerol synthesis. Glucose level inside the adipocytes determines whether fatty acids are released into the blood.(glycerol 3-phosphate needed for esterification is produced from glucose).</p>	<p>When the blood glucose level falls below the critical level, brain functions are affected. Brain adopts its metabolism to use ketone bodies as an alternative energy source.</p>	<p>The blood glucose spared by the brain is used by the red blood cells, which cannot use ketone bodies for energy because they have no mitochondria (So, citric acid cycle is absent).</p>

INTEGRATION OF METABOLISM - LIPIDS

Lipid metabolism : under well fed state			
Liver	Muscle	Adipose tissue	Brain
Excess dietary carbohydrates are converted to triacylglycerol (TG) in the liver. The TG is transported from the liver as VLDL. The lipoprotein lipase, which is needed for the hydrolysis of TG from VLDL and chylomicron is induced by insulin (excess glucose in blood stimulates insulin production). The fatty acids are taken up by adipose tissue and stored as TG in adipose tissues.	By the action of lipoprotein lipase on VLDL and chylomicron, free fatty acids are liberated and are used secondarily as fuel. When glucose is available it is used first for producing energy.	Fat is stored in the form of triacylglycerol, which is the major storage form in adipose tissue. During high carbohydrate diet, the transport of glucose into the adipocyte is stimulated by insulin. Some fatty acids that are incorporated into triacylglycerol are synthesized within the adipocyte from glucose. Adipocytes store TG coming from the liver.	Brain cannot use fatty acid as fuel because they are bound to albumin in plasma.
Lipid metabolism : under starvation			
Liver	Muscle	Adipose tissue	
When the glucose concentration is low, insulin level drops and the glucagon level increases, which stimulates the activity of hormone sensitive lipase that results in the raise in cellular cAMP level and lipolysis. The fatty acids obtained from adipose tissues are oxidized in the liver to produce energy. When the concentration of acetyl-CoA is more than the oxidative capacity of the Krebs cycle, the acetyl-CoA is shunted into ketone body synthesis. Ketone bodies are soluble form of fatty acid. They can be taken up by many organs and tissues, where	When the blood glucose concentration falls, fatty acids are taken up and oxidized by muscle. Fatty acids are mobilized from adipose tissue. This reduces the demand on blood glucose (it is maintained by glycogenolysis and gluconeogenesis in liver).	The starvation elevates epinephrine and glucagon levels, which stimulate the activity of hormone sensitive lipase. This hormone hydrolyzes triacylglycerol to free fatty acids (the level increases in plasma), which are taken to various tissues as fuel. The glycerol is taken to liver for the synthesis of glucose. As insulin level is reduced lipoprotein lipase activity is low. Consequently triacylglycerol of lipoprotein is not hydrolyzed to obtain free fatty acids. Humans and hibernating animals have brown fat, which generates heat rather than ATP production during oxidative phosphorylation.	

the ketone bodies serve as a good source of energy.		
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INTEGRATION OF METABOLISM -- PROTEINS

Protein metabolism : under well fed state		
Liver	Muscle	Brain
<p>It is the important site for amino acid metabolism. After a protein rich meal, the liver uses amino acids for the synthesis of proteins. The surplus amino acids are deaminated; the carbon skeleton is converted to pyruvate, other TCA cycle intermediates and acetyl-CoA. Pyruvate and TCA cycle metabolites can be converted to glucose through gluconeogenesis that can be released into the blood. These metabolites can also be oxidized to get energy. The acetyl CoA can be oxidized to provide energy or used for fatty acid biosynthesis. The liver contains urea cycle that converts toxic ammonia to non toxic urea. The carbon skeleton and nitrogen of amino acids are also used for the synthesis of nitrogen containing compounds such as heme, purines and pyrimidines.</p>	<p>In muscle the insulin released after a protein meal allows the uptake of amino acids and stimulates protein synthesis. The branched chain amino acids are used for muscle protein synthesis and are also metabolized to obtain energy.</p>	<p>The amino acids are used for the formation of glutamine and neurotransmitters.</p>
Protein metabolism : under starvation		
<p>Release of amino acids from skeletal muscle is stimulated due to reduced insulin and increased glucocorticoids. Liver takes up the amino acid to produce glucose.</p>	<p>During fasting degradation of muscle protein releases amino acids that are used by liver for glucose synthesis.</p> <p>Fasting muscle oxidizes branched chain amino acid to produce energy and glutamine.</p>	<p>Brain uses branched chain amino acid for energy. The amino acids also provide nitrogen for the synthesis of neurotransmitters.</p>