KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : METABOLIC PATHWAYS
SEMESTER : V
SUBJECT CODE : 15BCU501 **CLASS :** III B.Sc.(BC)

Programme outcome:

- This course enables the students to understand the synthesis and catabolism of major food stuffs namely carbohydrates, lipids, amino acids and nucleic acids. It also helps the students to correlate the interrelationship all the above said metabolism and the interconversion of major food stuffs within the body during various metabolic conditions.

Programme learning outcome:

- By learning this course, the students will understand the mechanism of conversion of food stuffs into energy.
- They also know the anabolic, catabolic and amphibolic pathways in metabolism and understand their significance
- Studying this subject will enable them to understand the utilization energy for various cellular process.
- This course helps them to understand the Clinical Biochemistry course which is pure based on the metabolism of macromolecules.

UNIT I

Introduction to intermediary metabolism

Introduction, overview of intermediary metabolism- the basic metabolic pathways, anabolic, catabolic and amphibolic pathways. Biological oxidation-oxidation, reduction equilibria; redox potential, enzymes and coenzymes involved in oxidation and reduction. ETC: Role of respiratory chain in energy capture. Oxidative phosphorylation- Mechanism of oxidative phosphorylation - Chemiosmotic theory, uncouplers of oxidative phosphorylation.

UNIT II

Carbohydrate metabolism

Introduction, fate of absorbed of carbohydrate, utilization of glucose, general processes of carbohydrate metabolism-glycolysis and citric acid cycle, glycogenesis, glycogenolysis and gluconeogenesis. Alternate pathways of carbohydrate metabolism-pentose phosphate pathway (HMP shunt), Glucuronic acid cycle and Glyoxylate cycle.

UNIT III

Metabolism of lipids

Introduction- Blood lipids and plasma lipoproteins- biomedical importance, fate of dietary lipids. Oxidation of Fatty acids: β- oxidation, α-oxidation and ω oxidation. Oxidation of fatty acids with odd numbers of carbon atoms. Biosynthesis of saturated fatty acids: Extra mitochondrial and microsomal system for elongation of fatty acids. Biosynthesis of Phospholipids: Phosphatidyl choline, Phosphatidyl ethanolamine, Phosphatidyl inositol and Phosphatidyl serine. Degradation of phospholipids, Biosynthesis of glycolipids, Biosynthesis of Cholesterol. Ketone bodies – formation, importance.

UNIT IV

Metabolism of protein and amino acids

Introduction, fate of dietary proteins, catabolism of aminoacid nitrogen-oxidative deamination; non-oxidative deamination, transamination-formation of ammonia, transport of ammonia, disposal of ammonia -urea cycle. Amino acid decarboxylation, Catabolism of carbon skeleton of aminoacids- glycine, tyrosine, phenyl alanine, glutamic acid and lysine.

UNIT V

Metabolism of purine and pyrimidine nucleotides

Introduction, biomedical importance, biosynthesis of purine and pyrimidine nucleotides, de novo synthesis of purines and pyrimidines, salvage pathways , catabolism of purines and pyrimidines.

Inter relationship of carbohydrate, protein and fat metabolism. TCA cycle as a central core in the inter relationships in metabolism and inter conversion of major food stuffs - Carbohydrate, fats and proteins.

TEXT BOOKS

Deb C., 2011, Fundamentals of Biochemistry, 9th edition New Central Book Agency, Calcutta.

Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell, 2012, Harper's Biochemistry, 29th edition, McGraw-Hill Medical, London.

Lehninger L, D.L. Nelson and M.M. Cox, 2012, Principles of Biochemistry, 6th edition. WH Freeman and Company, New York .

REFERENCES

Donald Voet, Jodith Voet and Charette.2012. Fundamentals of Biochemistry, 4th edition, John Wiley and Sons, Inc, New York.

Pamela C, Champ Richard and A. Harvey.2008. Biochemistry. Lipponcott Company Piladelphia.

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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME : Dr.K.DEVAKI
SUBJECT NAME : METABOLIC PATHWAYS SUB.CODE: 15BCU501
SEMESTER : V CLASS : III B.Sc (BC)

Sl. No	Duration of Period	Topics to be Covered	Books referred	Page No	Web page referred
Unit I: - INTRODUCTION TO INTERMEDIARY METABOLISM					
1.	1	Introduction – Overview of Intermediary metabolism	T1	365-366	
2.	1	The basic metabolic pathways- Anabolic and Catabolic pathways	T2	158-160	
3.	1	Amphibolic pathways	T2	159-160	W1
4.	1	Biological oxidation – oxidation and reduction, Redox potential	T2	116-117	
5.	1	Enzymes and Coenzymes involved in oxidation and reduction	T2	117-118	
6.	1	Enzymes and Coenzymes involved in oxidation and reduction (Continuation)	T2	118-119	
7.	1	Electron transport chain - complexes	T3	691-704	
8.	1	Test			
9.	1	Role of respiratory chain in energy capture	T2	126-128	
10.	1	Oxidative phosphorylation – Mechanism of oxidative phosphorylation	T3	708-711	
11.	1	Chemiosmotic theory	T3	712-714	
12.	1	Uncouplers of oxidative phosphorylation	T2	130-131	
13.	1	Revision			

14.	1	Possible QP discussion			
Total No of Hours planned for unit – I: 14					
Unit II: CARBOHYDRATE METABOLISM					
1	1	Introduction, Fate of absorbed carbohydrate	T2	161-162	
2	1	utilization of glucose	T2	162-163	
3	1	General process of carbohydrate metabolism	T3	521-522	
4	1	Glycolysis	T2	176-178	
5	1	Conversion of pyruvate to acetyl CoA	T2	179-181	
6	1	Citric acid cycle	T2	168-173	
7	1	Significance of TCA cycle	T2	173-175	
8	1	Test			
9	1	Glycogenesis	T1	237-239	
10	1	Glycogenolysis	T1	240-242	
11	1	Gluconeogenesis	T1	250-255	
12	1	Alternative pathway - HMP	T1	256-259	
13	1	Glucuronic acid	T1	259-260	
14	1	Glyoxalate cycle	T1	260-261	
15	1	Revision			
16	1	Possible QP discussion			
Total No of Hours planned for unit – II: 16					
Unit III: METABOLISM OF LIPIDS					
1	1	Introduction – Blood lipids - Biomedical importance	T2	250-254	
2	1	plasma lipoproteins-Biomedical importance	T2	254-258	
3	1	Fate of dietary lipids-Digestion of lipids	T3	632-633	
4	1	Absorbtion of lipids	T3	633-634	
5	1	Oxidation of fatty acids – β-oxidation,	T2	220-222	
6	1	α-oxidation and ω-oxidation and Energetics	T2	222-223	
7	1	Oxidation of fatty acid with odd number carbon atom	T2	224-225	
8	1	Test			
9	1	Biosynthesis of saturated fatty acid	T2	212-215	
10	1	Extra mitochondrial and microsomal system for elongation of fatty acid	T2	216-217	
11	1	Biosynthesis of phospholipids –	T2	240-241	

		Phosphatidyl choline, Phosphatidyl ethanolamine and Phosphatidyl serine.			
12	1	Biosynthesis of Phosphatidyl inositol and Degradation of phospholipids	T2	240-242	
13	1	Biosynthesis of glycolipids ,	T2	243-247	
14	1	Biosynthesis of Cholesterol	T2	266-270	
15	1	Ketone bodies – Formation and importance	T2	224-227	
16	1	Revision and Possible Questions discussion			
Total No of Hours planned for unit – III: 16					
Unit IV: METABOLISM OF PROTEIN AND AMINO ACIDS					
1	1	Introduction, fate of dietary proteins-Digestion	T1	330-331	
2	1	Fate of dietary proteins-Absorbtion	T1	331-332	
3	1	Catabolism of amino acid nitrogen-Oxidative deamination, Non-oxidative deamination	T1	332-333	
4	1	Transamination	T4	345-349	
5	1	Formation of ammonia, Transport of ammonia	T4	349-350	
6	1	Disposal of ammonia – Urea cycle	T4	350-354	
7	1	Test			
8	1	Amino acid decarboxylation	T4	144-147	
9	1	Catabolism of carbon skeleton of amino acid – glycine, tyrosine	T4	355-359	
10	1	Catabolism of carbon skeleton of amino acid –phenylalanine	T4	360-365	
11	1	Catabolism of carbon skeleton of amino acid – glutamic acid and lysine	T4	385-389	
12	1	Revision			
13		Possible QP discussion			
Total No of Hours planned for unit – IV: 13					
Unit V: METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES					
1	1	Introduction, Biomedical importance of purine and pyrimidines	T2	363-364	
2	1	Biosynthesis of purine nucleotide-Denovo synthesis	T2	364-366	

3	1	Biosynthesis of pyrimidine nucleotide- Denovo synthesis	T2	369-370	
4	1	Biosynthesis of purine and pyrimidine - Salvage pathway	T2	367-368	
5	1	Biosynthesis of pyrimidine - Salvage pathway	T2	370-371	
6	1	Test			
7	1	Catabolism of purines	T2	371-372	
8	1	Catabolism of pyrimidines	T2	373-375	
9	1	Inter relationship of carbohydrate, protein & fat metabolism	T2	279-282	
10	1	TCA cycle as a central core in the interrelationship of metabolism	T2	164-166	W2
11	1	Inter conversion of major food stuffs – Carbohydrate, fat & proteins	T2	154	W3
12	1	Revision			
13	1	Possible question discussion			
Total No of Hours planned for unit –V: 13					
PREVIOUS YEAR ESE QUESTION PAPER DISCUSSION					
1	1	Previous year ESE question paper Discussion			
2	1	Previous year ESE question paper Discussion			
3	1	Previous year ESE question paper Discussion			
Total No of Hours planned for ESE question paper discussion :03					
Total No of Hours planned for this course: 75					

Text book

T1 : Deb C., 2011, Fundamentals of Biochemistry, 9th ed. New central Book agency, Kolkata.

T2 : Robert K. Murray, Daryl K. Granner Peter A. Mayes Victor W. Rodwell, 2012, Harper's Biochemistry, 29th Ed, Mc Graw-Hill Medical, London.

T3: Lehninger L. Nelson. M M Cox, 2012. Principles of Biochemistry, 6th Edition, WH Freeman &company, New York

T4: Satyanarayana. U, 2008, Biochemistry, 3rd Edition, Books and Allied (P)Ltd, Kolkata.

Reference book

R1 : Chatterjea, M.N., Rana shinde, 2011, Text book of Medical Biochemistry, 8th Edition, Jaypee Brother medical publishers (P) Ltd. New Delhi.

Web page referred

W1- www.sciencedirect.com/topics/biochemistry-genetics-and-molecular.../amphibolic
W2- https://www.ncbi.nlm.nih.gov/books/NBK21163
W3- https://www.scribd.com/document/.../Interconversion-of-Carbohydrate-Protein-and-F.

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UNIT I-- COURSE MATERIAL

Introduction to intermediary metabolism

Introduction, overview of intermediary metabolism- the basic metabolic pathways, anabolic, catabolic and amphibolic pathways. Biological oxidation-oxidation, reduction equilibria; redox potential, enzymes and coenzymes involved in oxidation and reduction. ETC: Role of respiratory chain in energy capture. Oxidative phosphorylation- Mechanism of oxidative phosphorylation - Chemiosmotic theory, uncouplers of oxidative phosphorylation.

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UNIT-I: INTRODUCTION TO INTERMEDIARY METABOLISM

Introduction to Intermediary Metabolism

Generate energy from fuel molecules to feed all cells of the body. Some pathways are catabolic (break down molecules) and some are anabolic (produce fuel molecules. Metabolism comprises the entire set of chemical reactions that occur in a living organism that allow it to reproduce, develop, maintain its structure and respond to the environment. These chemical reactions form an intricate network of pathways and cycles in which the flow of reaction products (metabolites) is determined by many regulatory mechanisms. Traditionally, metabolism is subdivided into catabolism, the breaking down of complex molecules, and anabolism, processes related to the synthesis of complex organic substances.

According to the definition provided above, metabolism includes every cellular process, ranging from DNA replication to transcription and translation to enzyme function, and also involves the chemistry of small molecules in the cell. In this chapter, we will focus on intermediary metabolism, which describes all reactions concerned with the storage and generation of metabolic energy required for the biosynthesis of low-molecular weight compounds and energy storage compounds. In the intermediary metabolism pathway, the structure of each enzyme plays a crucial role in determining the specific properties of each reaction.

OVERVIEW OF INTERMEDIARY METABOLISM

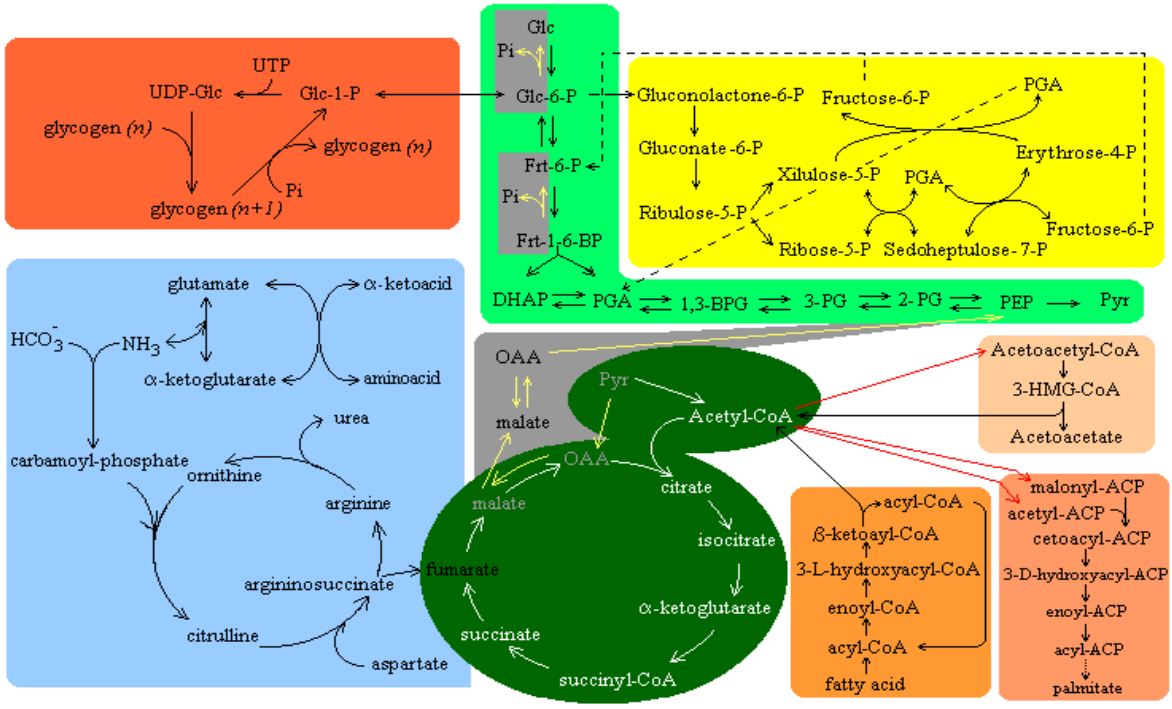
Intermediary metabolism is the fate of dietary carbohydrate, fat, and protein after digestion and absorption. Although admittedly intricate, all surgeons should be familiar with the chemical reactions by which food is converted to energy. Understanding the major biochemical pathways is a prerequisite to making use of the rapid and exciting expansion of medical knowledge directed at improving health beginning at the cellular level.

The major intermediary metabolites are glucose, fatty acids, glycerol, and amino acids. Glucose is metabolized to pyruvate and lactate by glycolysis. Aerobic metabolism allows conversion of pyruvate to acetyl coenzyme A (CoA). Acetyl CoA enters the citric acid cycle resulting in carbon dioxide, water, and reducing equivalents (a major source of adenosine triphosphate [ATP]). In the absence of oxygen, glycolysis ends in lactate. Glucose can be stored as or created from glycogen. Glucose can also enter the phosphogluconate pathway, where it is converted to reducing equivalents for fatty acid synthesis and ribose five-carbon sugars

important in nucleotide formation. Glucose can be converted into glycerol for fat formation and pyruvate for amino acid synthesis. Gluconeogenesis allows synthesis of glucose from lactate, amino acids, and glycerol. With regard to lipid metabolism, long-chain fatty acids arise from dietary fat or synthesis from acetyl CoA. Fatty acids can be oxidized to acetyl CoA by the process of β-oxidation or converted to acyl glycerols (fat) for storage as the main energy reserve. In addition to the fats noted previously, acetyl CoA can be used as a precursor to cholesterol and other steroids and in the liver can form the ketone bodies acetoacetate and 3-hydroxybutyrate, which are critical sources of energy during periods of starvation.

Proteins are degraded in two major ways: energy independent, usually in lysosomes, and energy requiring, usually through the ubiquitin pathway. Of amino acids generated in protein catabolism, about three fourths are reutilized for protein synthesis and one fourth are deaminated, with the resulting ammonia converted to urea. Amino acids may be divided into nutritionally essential and nonessential. Nonessential amino acids require fewer enzymatic reactions from amphibolic intermediates or essential amino acids. Each day, humans turn over 1% to 2% of total body protein.

THE BASIC METABOLIC PATHWAYS



Metabolism is the set of chemical rections that occur in a cell, which enable it to keep living, growing and dividing. Metabolic processes are usually classified as:

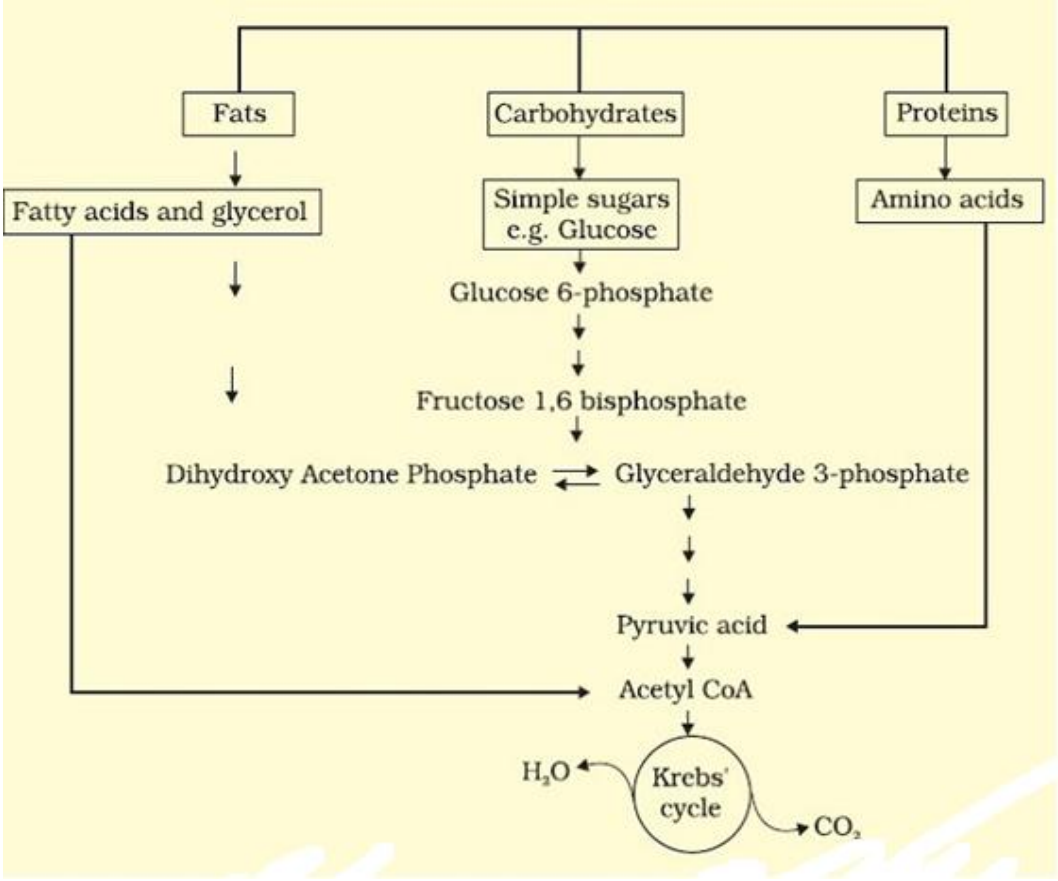
- **Catabolism** - obtaining energy and reducing power from nutrients.
- **Anabolism** - production of new cell components, usually through processes that require energy and reducing power obtained from nutrient catabolism.

  There is a very large number of metabolic pathways. In humans, the most important metabolic pathways are:
- **Glycolysis** - glucose oxidation in order to obtain ATP
- **Citric acid cycle** (Krebs' cycle) - acetyl-CoA oxidation in order to obtain GTP and valuable intermediates.
- **Oxidative phosphorylation** - disposal of the electrons released by glycolysis and citric acid cycle. Much of the energy released in this process can be stored as ATP.
- **Pentose phosphate pathway** - synthesis of pentoses and release of the reducing power needed for anabolic reactions.
- **Urea cycle** - disposal of NH4+ in less toxic forms
- **Fatty acid β-oxidation** - fatty acids breakdown into acetyl-CoA, to be used by the Krebs' cycle.
- **Gluconeogenesis** - glucose synthesis from smaller percursors, to be used by the brain.

ANABOLIC, CATABOLIC AND AMPHIBOLIC PATHWAY:

Metabolism is the sum of all of the chemical activities cells undergo throughout their lives. It is composed of two main subdivisions:

- Catabolism is the breakdown of biological molecules. Cells require nutrients containing energy stored in the carbon-to-carbon bonds of organic compounds, as well as inorganic substances that act as key components in all metabolic activities. Catabolic reactions serve to release energy and make substances in nutrients available for use.
- Anabolism (biosynthesis) is the building of new, novel organic compounds, utilizing the substances broken down by catabolic reactions. Anabolic pathways are necessary to synthesize metabolic and genetic materials, as well as those necessary for growth and reproduction. Both types of reactions occur through a series of enzyme-mediated steps called metabolic pathways. Linked anabolic and catabolic processes are called amphibolic pathways.



- The primary molecule used to store and deliver energy for all cell functions is adenosine triphosphate (ATP), a form of modified RNA nucleotide composed of the nitrogenous base adenine, the five-carbon sugar ribose and three phosphate groups, the last two of which are linked by high-energy covalent bonds.
- The term amphibolic is used to describe a biochemical pathway that involves both catabolism and anabolism. This term was proposed by B.Davis in 1961 to emphasise the dual metabolic role of such pathway. The citric acid cycle (The Krebs Cycle) is a good example of amphibolic pathway.
- The first reaction of the cycle, in which oxaloacetate (a four carbon compound) condenses with acetate (a two carbon compound) to form citrate (a six carbon compound) is typically anabolic. The next few reactions, which are intramolecular rearrangements, produce isocitrate.
- The following two reactions are typically catabolic. COO is lost in each step and succinate (a four carbon compound) is produced.There is an interesting and critical difference in the coenzymes used in catabolic and anabolic pathways; in catabolism

NAD^+ serves as an oxidizing agent when it is reduced to NADH. Whereas in anabolism the coenzyme NADPH serves as the reducing agent and is converted to its oxidized form $NADP^+$.

BIOLOGICAL OXIDATION

Biological oxidation is that oxidation which occurs in biological systems to produce energy.

Oxidation can occur by:

1-Addition of oxygen (less common)

2-Removal of hydrogen (common)

3-Removal of electrons (most common)

Electrons are not stable in the free State, so their removal form a substance (oxidation) must be accompanied by their acceptance by another substance (reduction) hence the reaction is called oxidation-reduction reaction or redox reaction and the involved enzymes are called oxido-reductases.

OXIDATION AND REDUCTION EQULIBRIA

It is the affinity of a substance to accept electrons i.e. it is the potential for a substance to become reduced. Hydrogen has the lowest redox potential (-0.42 volt), while oxygen has the highest redox potential (+0.82 volt). The redox potentials of all other substances lie between that of hydrogen and oxygen. Electrons are transferred from substances with low redox potential to substances with higher redox potential. This transfer of electrons is an energy yielding process and the amount of energy liberated depends on the redox potential difference between the electron donor and acceptor.

Oxidation:

Fe^{2+}_____ $Fe^{3+} + e^-$ removal of an electron

Reduction:

$Cu^{2+} + e^-$ _____ Cu^+ is the gain of the electron

The same principles apply to biological system

Respiration:

Respiration is a process by which cell derive energy in the form of ATP from controlled reaction of hydrogen with oxygen to form water.

The enzyme involve in the process of oxidation called oxidoreductases

Classified into four groups:

- Oxidases,
- Dehydrogenases
- Hydroperoxidases
- Oxygenases

OXIDATION-REDUCTION EQUILIBRIA

The usefulness of reduction potentials is greatly extended, however, by a thermodynamic relationship known as the Nernst equation, which makes it possible to calculate changes in half-cell potentials that will be produced by deviations from standard concentration conditions. In the reaction between zinc metal and copper(II) ion, standard conditions for zinc and copper metal require simply that both solids be present in contact with the solution; the E° values are not affected by either the total or proportionate amounts of the two metals. The calculation that the overall reaction is spontaneous by +1.10 volts is based on standard one mole per litre (1M) concentrations for aqueous zinc(II) ion (Zn2+[aq]) and aqueous copper(II) ion (Cu2+[aq]). Using the Nernst equation it is found that E° for the overall reaction will be +1.10 volts as long as both ions are present in equal concentrations, regardless of the concentration level.

On the other hand, if the ratio of the zinc (II) to copper (II) ion concentrations is increased, the reduction potential (E°) falls until, at a very high preponderance of zinc ion, E° becomes 0 volt. At this point, there is no net tendency for the reaction to proceed spontaneously in either direction. If the zinc (II) to copper (II) ion ratio is increased further, the direction of spontaneity reverses, and zinc ion spontaneously oxidizes copper metal. In practice, such high zinc (II) to copper (II) ion concentration ratios are unattainable, which means that the reaction can only be carried out spontaneously with copper (II) ion oxidizing zinc metal. Many reactions with E° values smaller than +1.10 volts under standard conditions can be carried out in either direction by adjusting the ratio of product and reactant concentrations. The point at which E° = 0 volt represents a state of chemical equilibrium. When chemical reactions are at equilibrium, the concentrations of the reagents do not change with time, since net reaction is not spontaneous in either direction. Measurements of half-cell potentials combined with Nernst-equation calculations are a powerful technique for determining the concentration conditions that correspond to chemical equilibrium.

Reaction rates

There are practical limitations on predictions of the direction of spontaneity for a chemical reaction, the most important arising from the problem of reaction rates. An analogy can be made with the simple physical system of a block on a sloping plane. Because of the favourable energy change, the block tends spontaneously to slide down, rather than up, the slope, and, at mechanical equilibrium, it will be at the bottom of the slope, since that is the position of lowest gravitational energy. How rapidly the block slides down is a more complex question, since it depends on the amount and kind of friction present. The direction of spontaneity for a chemical reaction is analogous to the downhill direction for a sliding block, and chemical equilibrium is analogous to the position at the bottom of the slope; the rate at which equilibrium is approached depends on the efficiency of the available reaction processes. Between zinc metal and aqueous copper (II) ion, the reaction proceeds without observable delay, but various other spontaneous redox processes proceed at imperceptibly slow rates under ordinary conditions.

Biological processes

A particularly significant illustration of the role of mechanisms in determining the rates of redox reactions concerns respiration, the central energy-producing process of life. Foodstuffs that are oxidized by molecular oxygen during respiration are quite unreactive with oxygen before ingestion. Such high-energy foods as grains and sugar can resist the atmosphere indefinitely but are rapidly converted to carbon dioxide and water through combination with oxygen during respiratory metabolism. The situation is exemplified by the behaviour of glucose at ambient temperatures.

The significance of the different rate behaviour of high-energy foods inside and outside the cell has been dramatized by Albert Szent-Györgyi, a Hungarian-born American biochemist and a pioneering researcher in the chemical mechanism of respiration:

At its opening the breakfast of the emperor was found unburned though it had been exposed to the action of oxygen during several thousand years at a temperature that was not very different from 37° C [98.6° F]. Had the king risen and consumed his breakfast, as he had anticipated doing, the food would have been oxidized in no time, that is to say the cells of the emperor would have made reactions take place that would not run spontaneously (from Albert V. Szent-Györgyi, On Oxidation, Fermentation, Vitamins, Health and Disease; the Williams and Wilkins Company, 1939). Living systems are able to use respiratory oxidation as an energy source only

because the same reactions are slow outside the cell. In return for providing an efficient mechanism for the oxidation of foods, the cell gains control over the disposition of the liberated chemical energy. Examples such as the chemistry of respiration make clear the importance of determining the rates and mechanisms of redox reactions. Often questions are difficult to answer even in regard to relatively simple reactions. It has been pointed out that many redox processes can be categorized as oxygen-atom-, hydrogen-atom-, or electron-transfer processes. These categories describe the net changes that are involved but provide no insight into the mechanisms of the reactions.

ENZYMES AND COENZYMES INVOLVE IN OXIDATION AND REDUCTION

Coenzymes are a type of cofactor. They are small organic molecules that bind tightly (prosthetic groups) or loosely (cosubstrates) to enzymes as they participate in catalysis. Coenzymes serve as carriers of several types of chemical groups. A prominent example of a coenzyme is nicotinamide adenine dinucleotide (NAD^+), which functions as a carrier of electrons in oxidation-reduction reactions. NAD^+ can accept a hydrogen ion (H^+) and two electrons (e^-) from one substrate, forming NADH. NADH can then donate these electrons to a second substrate, re-forming NAD^+. Thus, NAD^+ transfers electrons from the first substrate (which becomes oxidized) to the second (which becomes reduced)

The large quantity of NADH resulting from glycolysis, fatty acid oxidation, and the TCA cycle used to supply the energy for ATP synthesis via oxidative phosphorylation. Oxidation of NADH with phosphorylation of ADP to form ATP are processes supported by the mitochondrial electron transport assembly and ATP synthase, which are integral protein complexes of the inner mitochondrial membrane. The electron transport assembly is comprised of a series of protein complexes that catalyze sequential oxidation reduction reactions; some of these reactions are thermodynamically competent to support ATP production via ATP synthase provided a coupling mechanism, such as a common intermediate, is available. Proton translocation and the development of a transmembrane proton gradient provide the required coupling mechanism.

ELECTRON TRANSPORT CHAIN

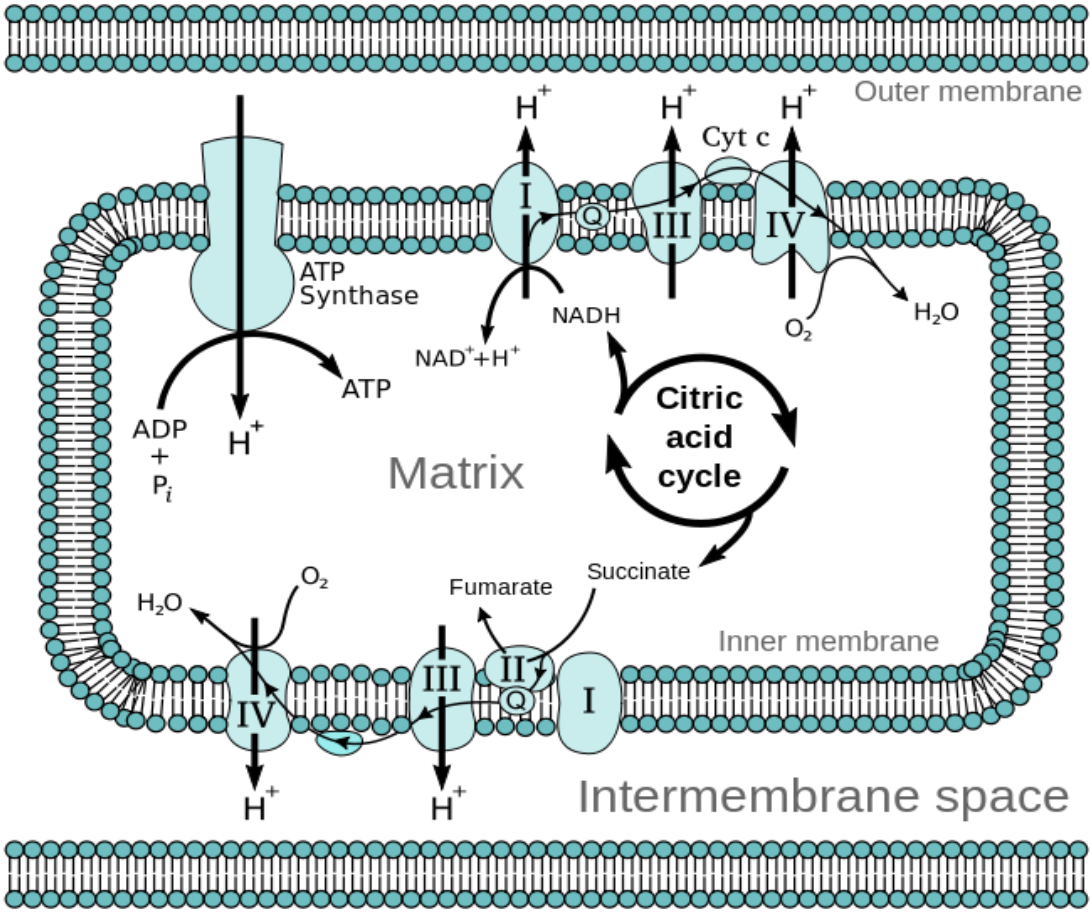
An electron transport chain (ETC) is a series of compounds that transfer electrons from electron donors to electron acceptors via redox reactions, and couples this electron transfer with the transfer of protons (H+ ions) across a membrane. This creates an electrochemical proton gradient that drives ATP synthesis, or the generation of chemical energy in the form of adenosine

triphosphate (ATP). Electron transport chains are used for extracting energy via redox reactions from sunlight in photosynthesis or, such as in the case of the oxidation of sugars, cellular respiration. In eukaryotes, an important electron transport chain is found in the inner mitochondrial membrane where it serves as the site of oxidative phosphorylation through the use of ATP synthase. It is also found in the thylakoid membrane of the chloroplast in photosynthetic eukaryotes. In bacteria, the electron transport chain is located in their cell membrane.

In chloroplasts, light drives the conversion of water to oxygen and $NADP^+$ to NADPH with transfer of H^+ ions across chloroplast membranes. In mitochondria, it is the conversion of oxygen to water, NADH to NAD+ and succinate to fumarate that are required to generate the proton gradient. Electron transport chains are major sites of premature electron leakage to oxygen, generating superoxide and potentially resulting in increased oxidative stress.

Electron transport chains in mitochondria

Most eukaryotic cells have mitochondria, which produce ATP from products of the citric acid cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and succinate pass through the electron transport chain to oxygen, which is reduced to water. The electron transport chain comprises an enzymatic series of electron donors and acceptors. Each electron donor passes electrons to a more electronegative acceptor, which in turn donates these electrons to another acceptor, a process that continues down the series until electrons are passed to oxygen, the most electronegative and terminal electron acceptor in the chain. Passage of electrons between donor and acceptor releases energy, which is used to generate a proton gradient across the mitochondrial membrane by actively “pumping” protons into the intermembrane space, producing a thermodynamic state that has the potential to do work. The entire process is called oxidative phosphorylation, since ADP is phosphorylated to ATP using the energy of hydrogen oxidation in many steps. A small percentage of electrons do not complete the whole series and instead directly leak to oxygen, resulting in the formation of the free-radical superoxide, a highly reactive molecule that contributes to oxidative stress and has been implicated in a number of diseases and aging.



Mitochondrial redox carriers

Energy obtained through the transfer of electrons (blue arrows) down the ETC is used to pump protons (red arrows) from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane (IMM) called $\Delta\Psi$. This electrochemical proton gradient allows ATP synthase (ATP-ase) to use the flow of H^+ through the enzyme back into the matrix to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Complex I (NADH coenzyme Q reductase; labeled I) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled Q), which also receives electrons from complex II (succinate dehydrogenase; labeled II). UQ passes electrons to complex III (cytochrome bc1 complex; labeled III), which passes them to cytochrome c (cyt c). Cyt c passes electrons to Complex IV (cytochrome c oxidase; labeled IV), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:

$NADH+H^+ \rightarrow$ Complex I $\rightarrow$ Q $\rightarrow$ Complex III $\rightarrow$ cytochrome c $\rightarrow$ Complex IV $\rightarrow O_2$

↑

Complex II

↑

Succinate

Complex I

In Complex I two electrons are removed from NADH and transferred to a lipid-soluble carrier, ubiquinone (Q). The reduced product, ubiquinol (QH_2), freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of superoxide.

The pathway of electrons occurs as follows:

NADH is oxidized to NAD^+, by reducing Flavin mononucleotide to $FMNH_2$ in one two-electron step. $FMNH_2$ is then oxidized in two one-electron steps, through a semiquinone intermediate. Each electron thus transfers from the $FMNH_2$ to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space.

Complex II

In Complex II additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD). Complex 2 is a parallel electron transport pathway to complex 1, but unlike complex 1, no protons are transported to the intermembrane space in this pathway. Therefore, the pathway through complex 2 contributes less energy to the overall electron transport chain process.

Complex III

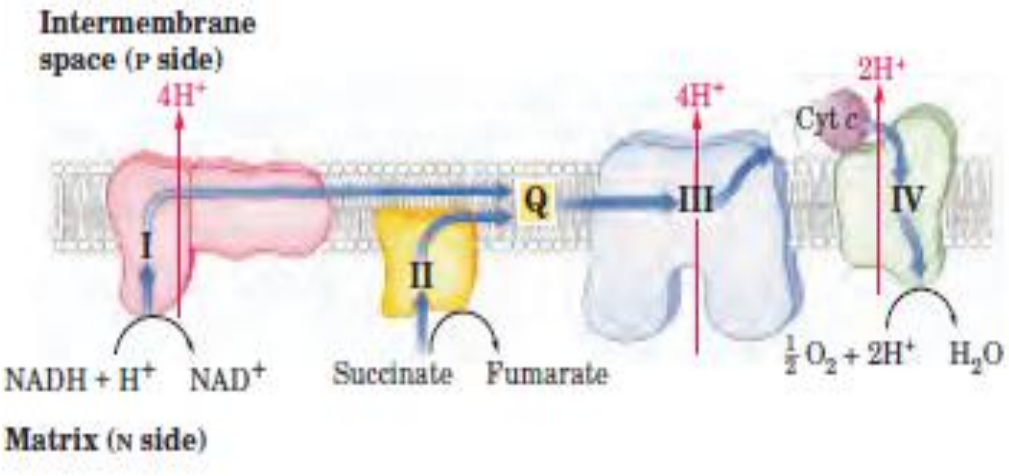
In Complex III, the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the QO site and sequentially transferred to two molecules of cytochrome c, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Qi site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by one quinol ($2H+2e^-$) oxidations at the Qo site to form one quinol (2H+2e-) at the Qi site. (in total six protons are translocated: two protons reduce quinone to quinol and two protons are released from two ubiquinol molecules).

$$QH_2 + 2 \text{ cytochrome c (FeIII)} + 2\ H^+_{in} \rightarrow Q + 2 \text{ cytochrome c (FeII)} + 4\ H^+_{out}$$

When electron transfer is reduced, Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

Complex IV

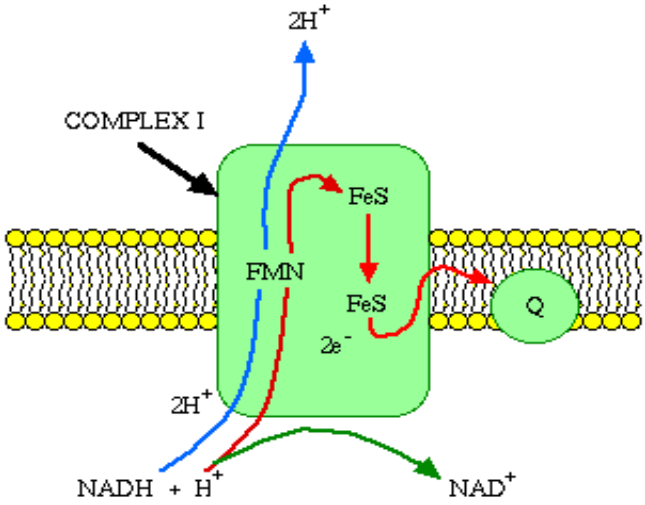
In Complex IV (cytochrome c oxidas) sometimes called cytochrome A3, four electrons are removed from four molecules of cytochrome c and transferred to molecular oxygen (O_2), producing two molecules of water. At the same time, four protons are removed from the mitochondrial matrix (although only two are translocated across the membrane), contributing to the proton gradient. The activity of cytochrome c oxidase is inhibited by cyanide.



Role of respiratory chain in energy capture

Embedded in the inner membrane are proteins and complexes of molecules that are involved in the process called electron transport. The electron transport system (ETS), as it is called,

accepts energy from carriers in the matrix and stores it to a form that can be used to phosphorylate ADP. Two energy carriers are known to donate energy to the ETS, namely nicotine adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). Reduced NAD carries energy to complex I (NADH-Coenzyme Q Reductase) of the electron transport chain. FAD is a bound part of the succinate dehydrogenase complex (complex II). It is reduced when the substrate succinate binds the complex.



When NADH binds to complex I, it binds to a prosthetic group called flavin mononucleotide (FMN), and is immediately re-oxidized to NAD. NAD is "recycled," acting as an energy shuttle. FMN receives the hydrogen from the NADH and two electrons. It also picks up a proton from the matrix. In this reduced form, it passes the electrons to iron-sulfur clusters that are part of the complex, and forces two protons into the intermembrane space. The obligatory forcing of protons into the intermembrane space is a key concept. Electrons cannot pass through complex I without accomplishing proton translocation. If prevent the proton translocation, it can be prevent electron transport.

Electron transport carriers are specific, in that each carrier accepts electrons from a specific type of preceding carrier. Electrons pass from complex I to a carrier (Coenzyme Q) embedded by itself in the membrane. From Coenzyme Q electrons are passed to a complex III which is associated with another proton translocation event. Note that the path of electrons is from Complex I to Coenzyme Q to Complex III. Complex II, the succinate dehydrogenase complex, is a separate starting point, and is not a part of the NADH pathway.

From Complex III the pathway is to cytochrome c then to a Complex IV (cytochrome oxidase complex). More protons are translocated by Complex IV, and it is at this site that oxygen binds, along with protons, and using the electron pair and remaining free energy, oxygen is reduced to water. Since molecular oxygen is diatomic, it actually takes two electron pairs and two cytochrome oxidase complexes to complete the reaction sequence for the reduction of oxygen. This last step in electron transport serves the critical function of removing electrons from the system so that electron transport can operate continuously.

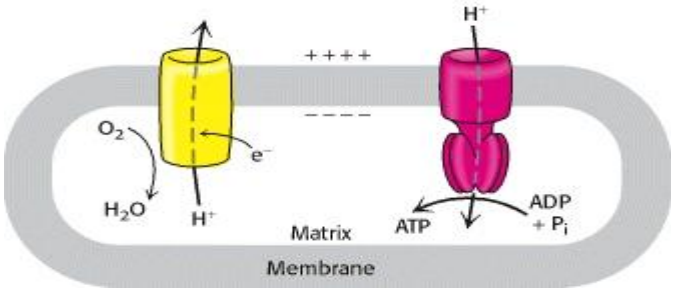
The reduction of oxygen is not an end in itself. Oxygen serves as an electron acceptor, clearing the way for carriers in the sequence to be reoxidized so that electron transport can continue. In your mitochondria, in the absence of oxygen, or in the presence of a poison such as cyanide, there is no outlet for electrons. All carriers remain reduced and Krebs products become out of balance because some Krebs reactions require NAD or FAD and some do not. However, you don't really care about that because you are already dead. The purpose of electron transport is to conserve energy in the form of a chemiosmotic gradient. The gradient, in turn, can be exploited for the phosphorylation of ADP as well as for other purposes. With the cessation of aerobic metabolism cell damage is immediate and irreversible. From succinate, the sequence is Complex II to Coenzyme Q to Complex III to cytochrome c to Complex IV. Thus there is a common electron transport pathway beyond the entry point, either Complex I or Complex II. Protons are not translocated at Complex II. There isn't sufficient free energy available from the succinate dehydrogenase reaction to reduce NAD or to pump protons at more than two sites.

OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation is the metabolic pathway in which the mitochondria in cells use their structure, enzymes, and energy released by the oxidation of nutrients to reform ATP. Although the many forms of life on earth use a range of different nutrients, ATP is the molecule that supplies energy to metabolism. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within the cell's intermembrane wall mitochondria, whereas, in prokaryotes, these

proteins are located in the cells' intermembrane space. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.



The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called electron transport. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase; this process is known as chemiosmosis. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor. Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and contributing to disease and, possibly, aging (senescence). The enzymes carrying out this metabolic pathway are also the target of many drugs and poisons that inhibit their activities.

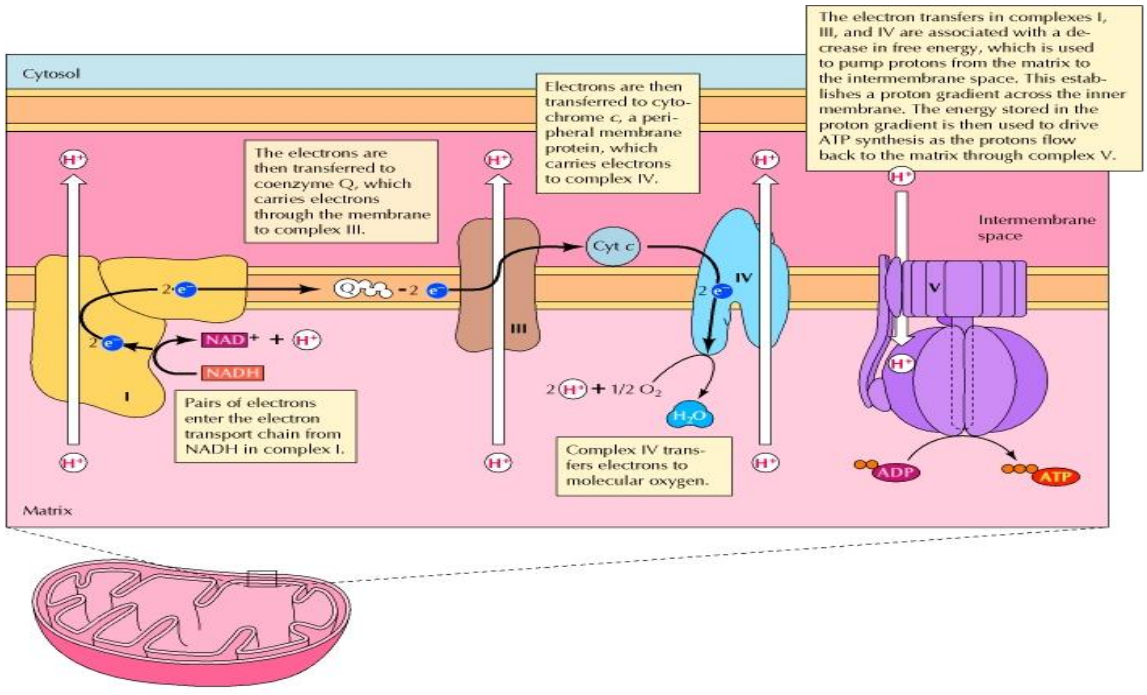
MECHANISM OF OXIDATIVE PHOSPHORYLATION

Most of the usable energy obtained from the breakdown of carbohydrates or fats is derived by oxidative phosphorylation, which takes place within mitochondria. For example, the breakdown of glucose by glycolysis and the citric acid cycle yields a total of four molecules of ATP, ten molecules of NADH, and two molecules of $FADH_2$. Electrons from NADH and $FADH_2$ are then transferred to molecular oxygen, coupled to the formation of an additional 32 to 34 ATP molecules by oxidative phosphorylation. Electron transport and oxidative phosphorylation are

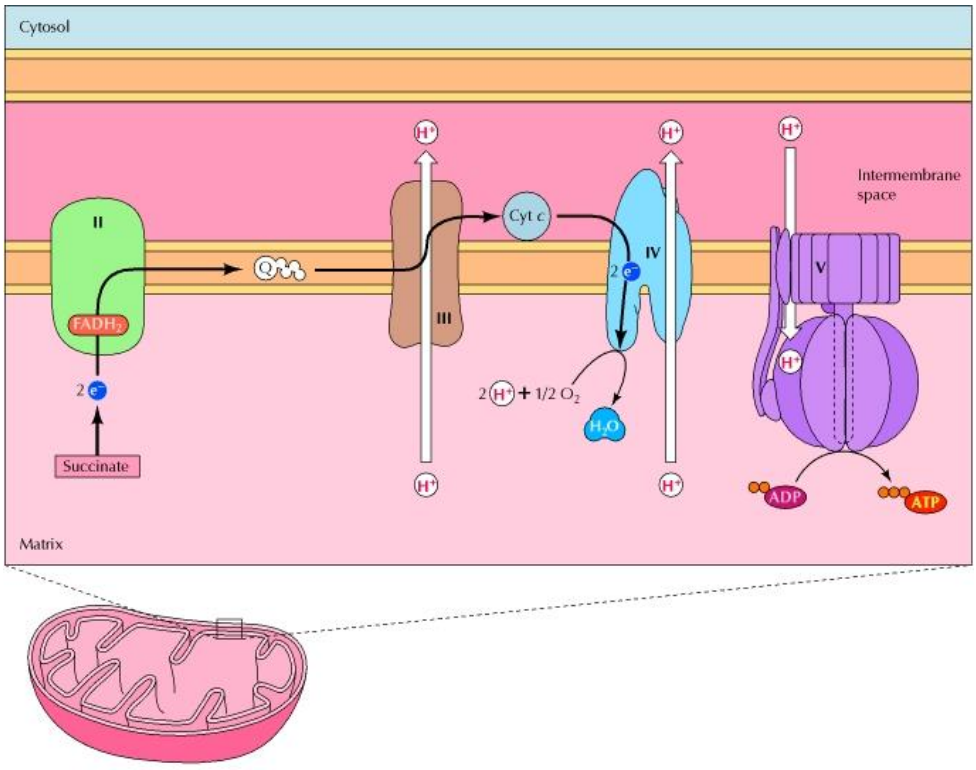
critical activities of protein complexes in the inner mitochondrial membrane, which ultimately serve as the major source of cellular energy.

During oxidative phosphorylation, electrons derived from NADH and $FADH_2$ combine with O_2, and the energy released from these oxidation/ reduction reactions is used to drive the synthesis of ATP from ADP. The transfer of electrons from NADH to O_2 is a very energy-yielding reaction. These carriers are organized into four complexes in the inner mitochondrial membrane. A fifth protein complex then serves to couple the energy-yielding reactions of electron transport to ATP synthesis.

Electrons from NADH enter the electron transport chain in complex I, which consists of nearly 40 polypeptide chains. These electrons are initially transferred from NADH to flavin mononucleotide and then, through an iron-sulfur carrier, to coenzyme Q-an energy-yielding process with $\Delta G^{\circ\prime}$ = -16.6 kcal/mol. Coenzyme Q is a small, lipid-soluble molecule that carries electrons from complex I through the membrane to complex III, which consists of about ten polypeptides. In complex III, electrons are transferred from cytochrome b to cytochrome c- an energy-yielding reaction with $\Delta G^{\circ\prime}$ = -10.1 kcal/mol. Cytochrome c, a peripheral membrane protein bound to the outer face of the inner membrane, then carries electrons to complex IV (cytochrome oxidase), where they are finally transferred to O2 ($\Delta G^{\circ\prime}$ = -25.8 kcal/mol).



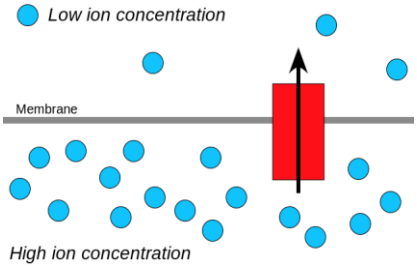
A distinct protein complex (complex II), which consists of four polypeptides, receives electrons from the citric acid cycle intermediate, succinate. These electrons are transferred to FADH2, rather than to NADH, and then to coenzyme Q. From coenzyme Q, electrons are transferred to complex III and then to complex IV as already described. In contrast to the transfer of electrons from NADH to coenzyme Q at complex I, the transfer of electrons from FADH2 to coenzyme Q is not associated with a significant decrease in free energy and, therefore, is not coupled to ATP synthesis. Consequently, the passage of electrons derived from FADH2 through the electron transport chain yields free energy only at complexes III and IV.



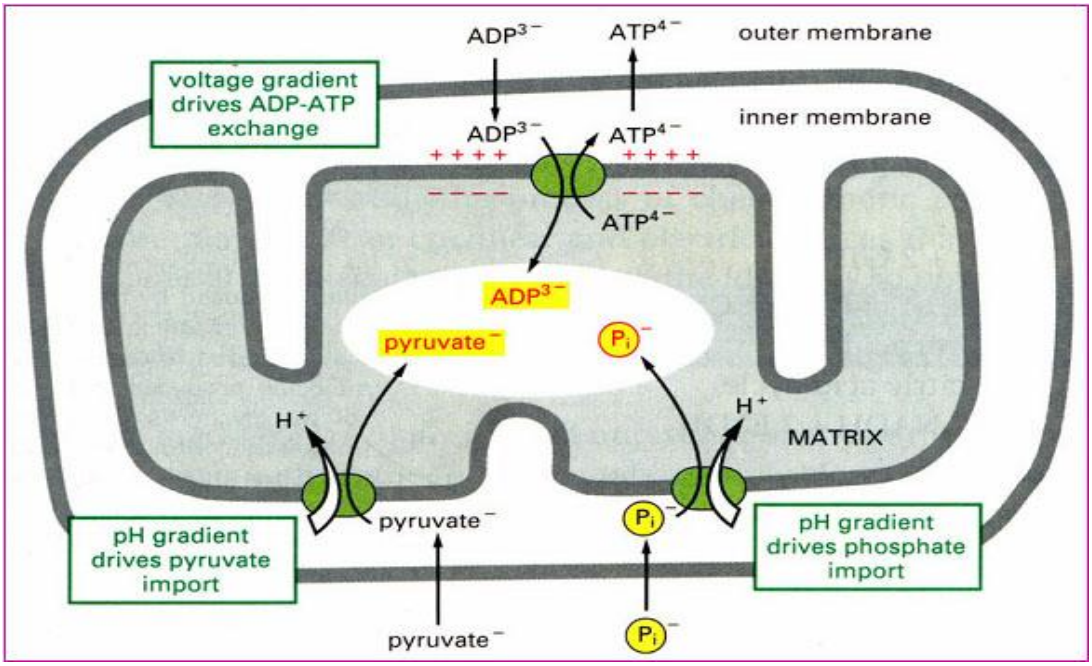
The free energy derived from the passage of electrons through complexes I, III, and IV is harvested by being coupled to the synthesis of ATP. Importantly, the mechanism by which the energy derived from these electron transport reactions is coupled to ATP synthesis is fundamentally different from the synthesis of ATP during glycolysis or the citric acid cycle. In the latter cases, a high-energy phosphate is transferred directly to ADP from the other substrate of an energy-yielding reaction. For example, in the final reaction of glycolysis, the high-energy phosphate of phosphoenolpyruvate is transferred to ADP, yielding pyruvate plus ATP.

CHEMIOSMOTIC THEORY

Chemiosmosis is the movement of ions across a selectively permeable membrane, down their electrochemical gradient. More specifically, it relates to the generation of ATP by the movement of hydrogen ions across a membrane during cellular respiration. An ion gradient has potential energy and can be used to power chemical reactions when the ions pass through a channel (red).



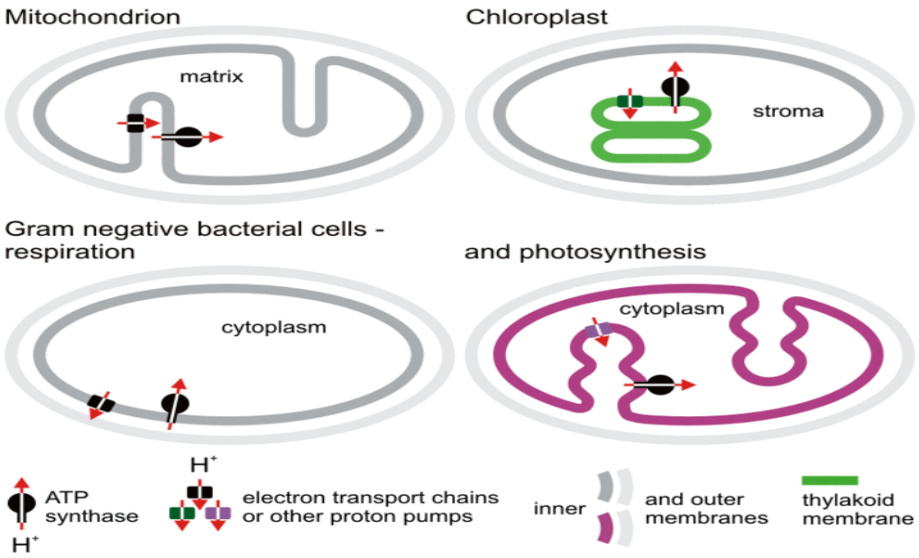
Hydrogen ions (protons) will diffuse from an area of high proton concentration to an area of lower proton concentration. Peter Mitchell proposed that an electrochemical concentration gradient of protons across a membrane could be harnessed to make ATP. He linked this process to osmosis, the diffusion of water across a membrane, which is why it is called chemiosmosis. ATP synthase is the enzyme that makes ATP by chemiosmosis. It allows protons to pass through the membrane and uses the kinetic energy to phosphorylate ADP, making ATP. The generation of ATP by chemiosmosis occurs in chloroplasts and mitochondria as well as in most bacteria and archaea.



The theory suggests essentially that most ATP synthesis in respiring cells comes from the electrochemical gradient across the inner membranes of mitochondria by using the energy of NADH and $FADH_2$ formed from the breaking down of energy-rich molecules such as glucose.Molecules such as glucose are metabolized to produce acetyl CoA as an energy-rich intermediate. The oxidation of acetyl CoA in the mitochondrial matrix is coupled to the reduction of a carrier molecule such as NAD and FAD. The carriers pass electrons to the electron transport chain (ETC) in the inner mitochondrial membrane, which in turn pass them to other proteins in the ETC. The energy available in the electrons is used to pump protons from the matrix across the inner mitochondrial membrane, storing energy in the form of a transmembrane electrochemical gradient. The protons move back across the inner membrane through the enzyme ATP synthase. The flow of protons back into the matrix of the mitochondrion via ATP synthase provides enough energy for ADP to combine with inorganic phosphate to form ATP. The electrons and protons at the last pump in the ETC are taken up by oxygen to form water.

In mitochondria

The complete breakdown of glucose in the presence of oxygen is called cellular respiration. The last steps of this process occur in mitochondria. The reduced molecules NADH and $FADH_2$ are generated by the Krebs cycle, glycolysis, and pyruvate processing. These molecules pass electrons to an electron transport chain, which uses the energy released to create a proton gradient across the inner mitochondrial membrane. ATP synthase then uses the energy stored in this gradient to make ATP. This process is called oxidative phosphorylation because oxygen is the final electron acceptor and the energy released by reducing oxygen to water is used to phosphorylate ADP and generate ATP.



In plants

The light reactions of photosynthesis generate energy by chemiosmosis. Light energy (photons) is received by the antenna complex of Photosystem 2, which excites a pair of electrons to a higher energy level. These electrons travel down an electron transport chain, causing H^+ to diffuse across the thylakoid membrane into the inter-thylakoid space. These H^+ are then transported down their concentration gradient through an enzyme called ATP-synthase, creating ATP by phosphorylation of ADP to ATP. The electrons from the initial light reaction reach Photosystem 1, then are raised to a higher energy level by light energy and then received by an electron receptor and reduce $NADP^+$ to $NADPH^+H$. The electrons from Photosystem 2 get replaced by the splitting of water, called "photolysis." Two water molecules must be split in order to gain 4 electrons.

Uncouplers of Oxidative Phosphorylation

Uncouplers can be defined as a *substance that uncouples phosphorylation of ADP from electron transfer.*

Uncoupling agents are compounds which dissociate the synthesis of ATP from the transport of electrons through the cytochrome system. This means that the electron transport continues to function, leading to oxygen consumption but phosphorylation of ADP is inhibited.

Below are few uncoupling agents,

1. 2,4-Dinitrophenol:

- A classic uncoupler of oxidative phosphorylation.
- The substance carries protons across the inner mitochondria membrane.
- In the presence of these uncouplers, electron transport from NADH to O_2 proceeds normally, but ATP is not formede by the mitochondria. ATP are because the proton motive force across the inner mitochondrial membrane is dissipated.
- DNA and other uncouplers are very useful in metabolic studies because of their specific effect on outside phosphorylation.

2. Dicoumarol (Vitamin.K analogue):

- Used as anticoagulant.

3. Calcium:

Transport of Ca^{+2} ion into mitochondria can cause uncoupling.

- Mitochondrial transport of Ca^{+2} is energetically coupled to oxidative phosphorylation.
- It is coupled with uptake of p^i
- When calcium is transported into mitochondria, electron transport can proceed but energy is required to pump the4 Ca^{+2} into the mitochondria. Hence, no energy is stored as ATP.

4. CCCP (Chloro carbonyl cyanide phenyl hydrazone):

- Most active uncoupler
- These lipid soluble substances can carry protons across the inner mitochondrial membrane.

5. Physiological un-couplers:

- Excessive thyroxin hormone
- EFA deficiency
- Long chain FA in brown adipose tissue
- Unconjugated hyperbilirubinaemia

Valinomycin:

- This is the example to Ionophore of oxidative phosphorylation.
- Produced by a type of streptomyces
- It is a repeating macrocyclic molecule made up of four kinds of residues (L-lactate, L-Valine, D-hydroxyisovalarate and D-Valine) taken 3 times.

- Transports K^+ from the cytosol into matrix and H^+ from matrix to cytosol, thereby decreasing the proton gradient.

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III B.Sc BIOCHEMISTRY
BATCH: 2015 - 2018

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

SUBJECT: METABOLIC PATHWAYS **SUBJECT CODE: 15BCU501** **UNIT: I**

S. No	Question	Option A	Option B	Option C	Option D	Answer
1.	Enzymes catalyzing electron transport are present mainly in the	Ribosomes	Endoplasmic reticulum	Lysosomes	Inner mitochondrial membrane	Inner mitochondrial membrane
2.	The power house of the cell is	Nucleus	Cell membrane	Mitochondria	Lysosomes	Mitochondria
3.	Starch is a	Polysaccharide	Monosaccharide	Disaccharide	Trisaccharide	Polysaccharide
4.	Oxidation is a	Gain of the electron	Removal of an electron	Gain of an hydrogen ion	Removal of an oxygen	Removal of an electron
5.	Reduction is a	Removal of an oxygen	Gain of an oxygen	Gain of the electron	Removal of an electron	Gain of the electron
6.	Electron transport and phosphorylation can be uncoupled by compounds that increase the permeability of the inner mitochondrial membrane to	Electrons	Protons	Uncouplers	Couplers	Protons
7.	The function of an electron in the electron transport chain is	To transfer energy from complex II to complex I	To pump hydrogen ions using complex II	pump protons against their concentration	phosphate when ATP is synthesized	protons against their concentration gradient
8.	In ETC, succinate dehydrogenase is also denoted as	Complex I	Complex II	Complex III	Complex IV	Complex II
9.	Coenzyme Q is involved in electron transport as	Directly to O_2	A water-soluble electron donor	Covalently attached cytochrome cofactor	A lipid-soluble electron carrier	A lipid-soluble electron carrier
10.	Which of the following is a coenzyme associated with cellular respiration?	NAD^+	O_2	FAD	NAD and FAD	NAD and FAD
11.	The _____ connects glycolysis to the Citric Acid cycle.	Electron transport system	Transition reaction	Cristae	Mitochondrial intermembrane	Transition reaction
12.	When electrons are removed from pyruvate in the transition reaction, they are accepted by _____	acetyl Co-A	FAD^+	NAD^+	ATP	NAD^+
13.	The electron transport system is located in the _____	Stroma	Matrix	Cytosol	Cristae	Cristae
14.	When O_2 is reduced during the electron transport system, _____ is produced	H_2O	CO_2	ADP	Glucose	H_2O
15.	The final electron acceptor in the electron transport system is _____	$FADH_2$	O_2	Coenzyme Q	Cytochrome b	O_2
16.						

16.	Oxidative phosphorylation occurs in	Mitochondria	Nucleus	Cell Membrane	Ribosomes	Mitochondria
17.	Electrons in electron transport chains reduces oxygen in to	Carbondioxide	Water	Carbonmonoxide	Ozone	Water
18.	by oxidation of NADH and $FADH_2$ is called	Phosphorylation	Electron transport chain	Dehydrogenation	Oxidative phosphorylation	Electron transport chain
19.	Electron transport chain consists of how many complexes	7	5	3	4	4
20.	Major components of electron transport chain are arranged in order of redox potential which is	Decreasing	Increasing	Variable	Alternatively increasing and decreasing	Increasing
21.	Cytochromes are enzymes which function as electron transfer agent in	Hydrolysis	Conjugation reaction	Transamination	Oxidation and reduction	Oxidation and reduction
22.	Oxidative phosphorylation is a process for	Phosphorylation of glucose	Generating creatine phosphate	Generating ATP	Utilizing ATP	Generating ATP
23.	Catabolism is	Breakdown of biological molecules	Synthesis of biological molecules	Conversion of biological molecules	Utilizing of biological molecules	Breakdown of biological molecules
24.	Anabolism is	Utilization of molecules	Breakdown of molecules	Biosynthesis of molecules	Conversion of molecules	Biosynthesis of molecules
25.	Amphibolic is	Catabolism	Anabolism	Metamorphisam	Both catabolism and anabolism	Both catabolism and anabolism
26.	Molecule generated in oxidative phase of pentose phosphate pathway is	NADP	NADPH	ADP	ATP	NADPH
27.	Number of phases in pentose phosphate pathway are	2	4	6	8	2
28.	Primary role of pentose phosphate pathway is	Catabolic	Anabolic	Both A and B	Amphibolic	Anabolic
29.	Pentose phosphate pathway is parallel to	Glycolysis	Gluconeogenesis	Fermentation	Respiration	Glycolysis
30.	Pentose phosphate pathway is also termed as	Glycolysis	Gluconeogenesis	Phosphogluconate pathway	Glycogenolysis	Phosphogluconate pathway
31.	Uncoupling of mitochondrial oxidative phosphorylation	Allows continued mitochondrial ATP formation, but halts O_2 consumption	Halts all mitochondrial metabolism	Halts mitochondrial ATP formation, but allows continued O_2 consumption	Slows the conversion of glucose to pyruvate by glycolysis	Halts mitochondrial ATP formation, but allows continued O_2 consumption
32.	During oxidative phosphorylation, the proton motive force that is generated by electron transport is used to	Create a pore in the inner mitochondrial membrane	Generate the substrates for the ATP synthase	Induce a conformational change in the ATP synthase	Oxidize NADH to NAD^+	Induce a conformational change in the ATP synthase
33	What is the most common electron carrier in biological systems?	FAD	Coenzyme A	NAD	NADP	NAD

34.	What is complex is not found in the electron transport system?	NADH dehydrogenase	Flavoproteins	NADPH dehydrogenase	Coenzyme Q	NADPH dehydrogenase
35.	The coupling of ATP synthesis to electron transport is known as	Oxidative phosphorylation	Chemiosmosis	ATP synthesis	Proton motive force	Oxidative phosphorylation
34	Which of the following drives the synthesis of ATP by ATP synthase in oxidative phosphorylation?	Distribution of electric potential across a membrane	Distribution of Cytochrome oxidase	Distribution of NADH	Distribution of FADH	Distribution of electric potential across a membrane
35.	Which is not a metabolic intermediate used in amphibolic pathways?	Glyceraldehyde-3-phosphate	Fructose-1,6-bisphosphate	Acetyl CoA	Oxaloacetic acid	Fructose-1,6-bisphosphate
36.	The compound having the lowest redox potential amongst the following is	Hydrogen	NAD	Cytochrome b	Cytochrome a	Hydrogen
37	The compound having the highest redox potential amongst the following is	Coenzyme Q	NAD	Cytochrome c	Cytochrome b	Cytochrome c
38	Superoxide radicals can be detoxified by	Cytochrome c	Cytochrome b	Cytochrome a	Coenzyme Q	Cytochrome c
39	Which of the following is a coenzyme?	FAD	Ca^{2+}	Mg^{2+}	CO_2	FAD
40	An exergonic reaction is one in which	Electrons are added to a molecule	Electrons are removed from a molecule	The products have more free energy than the reactants	The reactants have more free energy than the products	The reactants have more free energy than the products
41	The main endergonic reaction that is driven by most of the body's exergonic reactions is the	Oxidation of $FADH_2$	Synthesis of ATP	Reduction of NAD	Hydrolysis of ATP	Synthesis of ATP
42.	The "universal energy carrier" is	FAD	$FADH_2$	Glucose	Adenosine triphosphate	Adenosine triphosphate
43	If molecule A accepts electrons from molecule B, molecule A is	Reduced agent	A reducing agent	An oxidizing agent	An exergonic agent	An oxidizing agent
44	Any oxidation reaction must be coupled to	The synthesis of ATP	The availability of oxygen	An exergonic reaction	A reduction reaction	A reduction reaction
45.	If a molecule accepts a hydrogen atom, it becomes	Hydrolyzed	Dehydrated	Oxidized	Reduced	Reduced
46	Nicotinamide adenine dinucleotide (NAD) is	A vitamin	An oxidizing agent	A reducing agent	A coenzyme	A coenzyme
47	In the electron transport chain, the hydrogen ions enter the inner compartment of mitochondria through	ATP synthase				

	special channels formed by		Coenzyme A	Acetyl CoA	Oxygen	ATP synthase
48	glucose results in _____ ATP molecules	38	36	32	39	38
49	Which process produces both NADH and $FADH_2$?	The citric acid cycle	Glycolysis	The electron transport system	Fermentation	The citric acid cycle
50.	Which process promptly involves with chemiosmotic phosphorylation?	The citric acid cycle	The electron transport system with ATP synthase	Glycolysis	Fermentation	The electron transport system with ATP synthase
51.	Which of these pairs of processes are anaerobic?	Fermentation and glycolysis	Fermentation and the citric acid cycle	Glycolysis and the citric acid cycle	The citric acid cycle and the electron transport system	Fermentation and glycolysis
52.	During anerobic glycolysis glucose is degraded to produce	Lactic acid	Pyruvic acid	Citric acid	Both A and B	Lactic acid
53.	ETC is located in the	Outer mitochondrial membrane	Inner mitochondrial membrane	Mitochondrial matrix	Nucleus	Inner mitochondrial membrane
55	Coenzyme Q catalyzes electron transport between	FADH and cytochrome b	It is the last member in the ETC	NADH and ubiquinone	Cytochrome Q and cytochrome c	FADH and cytochrome b
56.	The enzymes of ETC belong to the following classes except	Oxidases	Dehydrogenases	Peroxidases	Reductases	Peroxidases
58	Which of the electron carriers is soluble and mobile	CoQ	Cytochrome c	Cytochrome a	Cytochrome b	CoQ
59.	During chemiosmosis in aerobic respiration, protons are pumped	Out of the outer membrane of mitochondria	Out of the mitochondria into the cell cytoplasm	Out of the mitochondrial matrix into intermembrane space of the mitochondria	Out of the cell cytoplasm into the matrix of the mitochondria	Out of the mitochondrial matrix into intermembrane space of the mitochondria
60.	The final electron acceptor in lactic acid fermentation is:	Phosphoenol pyruvate	Pyruvate	Malate	Lactic acid	Pyruvate

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DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **METABOLIC PATHWAYS**
SEMESTER : **V**
SUBJECT CODE : **15BCU501** **CLASS** : **III B.Sc.BC**

UNIT II-- COURSE MATERIAL

Carbohydrate metabolism

Introduction, fate of absorbed of carbohydrate, utilization of glucose, general processes of carbohydrate metabolism-glycolysis and citric acid cycle, glycogenesis, glycogenolysis and gluconeogenesis. Alternate pathways of carbohydrate metabolism-pentose phosphate pathway (HMP shunt), Glucuronic acid cycle and Glyoxylate cycle

TEXT BOOKS

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UNIT-II: CARBOHYDRATE METABOLISM

Introduction

Carbohdrate Digestion

The two digestible carbohydrates are starches and sugars, and both of these carbohydrates are digested, or broken down into their most elementary form, along the gastrointestinal tract. Amylase, an enzyme which breaks apart starches, is found in the mouth and in the small intestine. Similarly, the three major enzymes which break apart sugars -- sucrase, maltase and lactase -- are also found in the mouth and in the small intestine. Once these digested starches and sugars begin to move through the small intestine, they are able to be absorbed.

Carbohydrate Absorption

Once carbohydrates are broken down into their simplest forms, they are quickly absorbed along the upper and lower parts of the small intestine. Small, finger-like projections, called villi, absorb the carbohydrates, then they are transferred to the blood stream and carried to muscles and the liver.

Carbohydrate Elimination

When carbohydrates are not fully digested or absorbed, they are eliminated from the body. Dietary fiber is one of the carbohydrates which humans cannot digest, thus dietary fiber is the most commonly excreted type of carbohydrate. In addition, lactose, a type of sugar, can also be excreted if an individual lacks the proper enzymes to digest this carbohydrate. All undigested carbohydrates move from the small intestine, where absorption would normally occur, to the large intestine and the colon, where elimination finally occurs.

Fate of absorbed carbohydrate

Carbohydrates can be chemically divided into complex and simple. Simple carbohydrates consist of single or double sugar units (monosaccharides and disaccharides, respectively). Sucrose or table sugar (a disaccharide) is a common example of a simple carbohydrate. Complex carbohydrates contain three or more sugar units linked in a chain, with most containing hundreds to thousands of sugar units. They are digested by enzymes to release the simple sugars. Starch, for example, is a polymer of glucose units and is typically broken down to glucose. Cellulose is

also a polymer of glucose but it cannot be digested by most organisms. Some bacteria that produce enzymes for cellulose live inside the gut of some mammals such as cows, and when cows eat plants, the cellulose is broken down by the bacteria and some of it is released into the gut.

Studies suggest that complex carbohydrates are indeed digested more slowly, and choosing complex carbohydrates may produce more stable blood sugar. The blood glucose rises and falls more slowly after the consumption of bread compared to sugars or fruit, and the carbohydrate oxidation rate rises more slowly after the consumption of bread rather than sucrose following an overnight fast. However, many complex carbohydrates such as bread, rice, and potatoes have glycemic indices that are similar to or higher than simple carbohydrates such as sucrose. Sucrose has a glycemic index lower than expected because the sucrose molecule is half fructose, which has little effect on blood glucose. The glycemic index is, therefore, a better predictor of a carbohydrate's effect on blood glucose than the classification of simple and complex.

Carbohydrates are a superior short-term fuel for organisms because they are simpler to metabolize than fats or those amino acids (components of proteins) that can be used for fuel. In animals, the most important carbohydrate is glucose. The concentration of glucose in the blood is used as the main control for the central metabolic hormone, insulin. Starch, and cellulose in a few organisms, both being glucose polymers, are disassembled during digestion and absorbed as glucose. Some simple carbohydrates have their own enzymatic oxidation pathways, as do only a few of the more complex carbohydrates. The disaccharide lactose, for instance, requires the enzyme lactase to be broken into its monosaccharides components; many animals lack this enzyme in adulthood.Carbohydrates are typically stored as long polymers of glucose molecules with glycosidic bonds for structural support (e.g. chitin, cellulose) or for energy storage (e.g. glycogen, starch). However, the strong affinity of most carbohydrates for water makes storage of large quantities of carbohydrates inefficient due to the large molecular weight of the solvated water-carbohydrate complex. In most organisms, excess carbohydrates are regularly catabolised to form acetyl-CoA, which is a feed stock for the fatty acid synthesis pathway; fatty acids, triglycerides, and other lipids are commonly used for long-term energy storage. The hydrophobic character of lipids makes them a much more compact form of energy storage than hydrophilic carbohydrates. However, animals, including humans, lack the necessary enzymatic machinery and so do not synthesize glucose from lipids, though glycerol can be converted to glucose.

Utilization of glucose

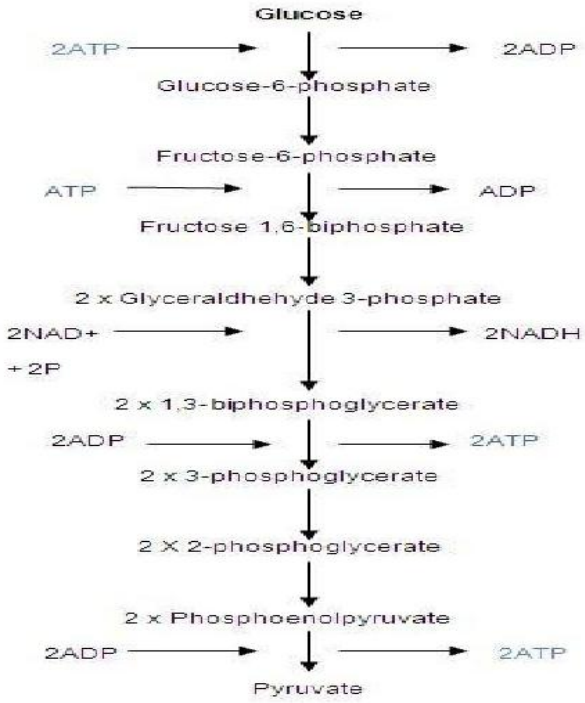
Carbohydrate metabolism denotes the various biochemical processes responsible for the formation, breakdown and inter conversion of carbohydrates in living organisms. The most important carbohydrate is glucose, a simple sugar (monosaccharide) that is metabolized by nearly all known organisms.Glucose and other carbohydrates are part of a wide variety of metabolic pathways across species: plants synthesize carbohydrates from carbon dioxide and water by photosynthesis storing the absorbed energy internally, often in the form of starch or lipids. Plant components are consumed by animals and fungi, and used as fuel for cellular respiration. Energy obtained from metabolism (e.g. oxidation of glucose) is usually stored temporarily within cells in the form of ATP. Organisms capable of aerobic respiration metabolize glucose and oxygen to release energy with carbon dioxide and water as byproducts

General Process of Carbohydrate Metabolism

All carbohydrates share a general formula of approximately CnH2nOn; glucose is $C_6H_{12}O_6$. Monosaccharides may be chemically bonded together to form disaccharides such as sucrose and longer polysaccharides such as starch and cellulose. Carbon fixation, or photosynthesis, in which CO2 is reduced to carbohydrate.

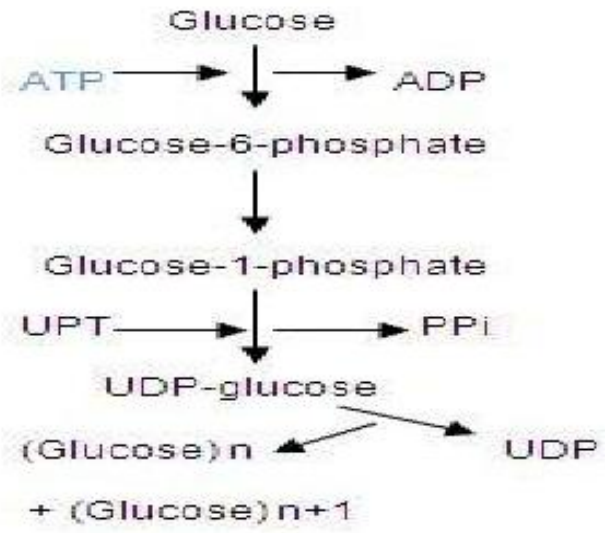
Glycolysis - the oxidation metabolism of glucose molecules to obtain ATP and pyruvate

Pyruvate from glycolysis enters the Krebs cycle, also known as the citric acid cycle, in aerobic organisms after moving through pyruvate dehydrogenase complex.

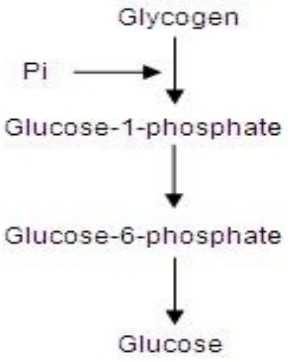


The pentose phosphate pathway, which acts in the conversion of hexoses into pentoses and in NADPH regeneration. NADPH is an essential antioxidant in cells which prevents oxidative damage and acts as precursor for production of many biomolecules.

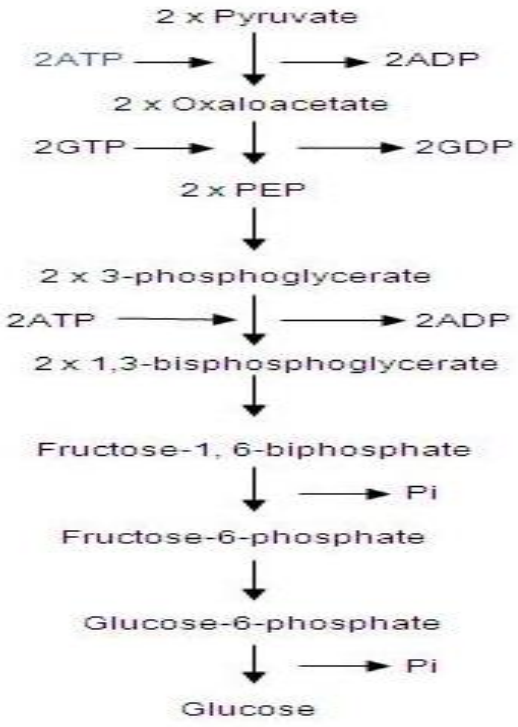
Glycogenesis - the conversion of excess glucose into glycogen as a cellular storage mechanism; this prevents excessive osmotic pressure buildup inside the cell



Glycogenolysis - the breakdown of glycogen into glucose, which provides a glucose supply for glucose-dependent tissues.



Gluconeogenesis - de novo synthesis of glucose molecules from simple organic compounds. An example in humans is the conversion of a few amino acids in cellular protein to glucose.



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

GLYCOLYSIS

Glycolysis is an almost universal pathway for extraction of the energy available from carbohydrates, shared among prokaryotes and eukaryotes, aerobes and anaerobes alike. In anaerobes, glycolysis is the only significant source of energy from carbohydrates. In aerobic

organisms, considerably more energy can be harvested downstream from glycolysis in the citric acid cycle. Glycolysis produces energy in the form of ATP and NADH. The glycolytic pathway consists of 10 enzyme-catalyzed steps. During glycolysis, glucose, a six-carbon carbohydrate, is oxidized to form two molecules of pyruvate, a three-carbon molecule. For each glucose molecule metabolized, the pathway produces two molecules of ATP and two molecules of NADH.

Glycolysis is not isolated from other metabolic pathways. Other molecules besides glucose can enter at a few points along the glycolytic pathway. For example, the product of glycogen breakdown, glucose-6-phosphate, can enter the glycolytic pathway at the second step. Glyceraldehyde-3-phosphate, which is produced by photosynthesis, is also a glycolytic intermediate, so it can be directed from this anabolic pathway into glycolysis when energy is needed. Additionally, intermediates can be drawn out of the glycolytic pathway when energy levels are high, for use in biosynthetic pathways. For instance, during active energy production pyruvate, the product of glycolysis, enters the citric acid cycle, but when energy is not needed pyruvate serves as a substrate in amino acid synthesis.

Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates. The first step in glycolysis is phosphorylation of glucose by a family of enzymes called hexokinases to form glucose 6-phosphate (G6P). This reaction consumes ATP, but it acts to keep the glucose concentration low, promoting continuous transport of glucose into the cell through the plasma membrane transporters. In addition, it blocks the glucose from leaking out – the cell lacks transporters for G6P, and free diffusion out of the cell is prevented due to the charged nature of G6P. Glucose may alternatively be formed from the phosphorolysis or hydrolysis of intracellular starch or glycogen. In animals, an isozyme of hexokinase called glucokinase is also used in the liver, which has a much lower affinity for glucose (Km in the vicinity of normal glycemia), and differs in regulatory properties. The different substrate affinity and alternate regulation of this enzyme are a reflection of the role of the liver in maintaining blood sugar levels.cofactors: $Mg2^{+}$.

G6P is then rearranged into fructose 6-phosphate (F6P) by glucose phosphate isomerase. Fructose can also enter the glycolytic pathway by phosphorylation at this point.The change in structure is an isomerization, in which the G6P has been converted to F6P. The reaction requires an enzyme, phosphohexose isomerase, to proceed. This reaction is freely reversible under normal

cell conditions. However, it is often driven forward because of a low concentration of F6P, which is constantly consumed during the next step of glycolysis. Under conditions of high F6P concentration, this reaction readily runs in reverse. This phenomenon can be explained through Le Chatelier's Principle. Isomerization to a keto sugar is necessary for carbanion stabilization in the fourth reaction step.

The energy expenditure of another ATP in this step is justified in 2 ways: The glycolytic process (up to this step) is now irreversible, and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase 1 (PFK-1) is coupled to the hydrolysis of ATP, an energetically favorable step, it is, in essence, irreversible, and a different pathway must be used to do the reverse conversion during gluconeogenesis. This makes the reaction a key regulatory point (see below). This is also the rate-limiting step. Furthermore, the second phosphorylation event is necessary to allow the formation of two charged groups (rather than only one) in the subsequent step of glycolysis, ensuring the prevention of free diffusion of substrates out of the cell.

The same reaction can also be catalyzed by pyrophosphate-dependent phosphofructokinase (PFP or PPi-PFK), which is found in most plants, some bacteria, archea, and protists, but not in animals. This enzyme uses pyrophosphate (PPi) as a phosphate donor instead of ATP. It is a reversible reaction, increasing the flexibility of glycolytic metabolism. A rarer ADP-dependent PFK enzyme variant has been identified in archaean species.

Cofactors: $Mg2^{+}$.

Destabilizing the molecule in the previous reaction allows the hexose ring to be split by aldolase into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde. There are two classes of aldolases: class I aldolases, present in animals and plants, and class II aldolases, present in fungi and bacteria; the two classes use different mechanisms in cleaving the ketose ring.

Electrons delocalized in the carbon-carbon bond cleavage associate with the alcohol group. The resulting carbanion is stabilized by the structure of the carbanion itself via resonance charge distribution and by the presence of a charged ion prosthetic group. Triosephosphate isomerase rapidly interconverts dihydroxyacetone phosphate with glyceraldehyde 3-phosphate (GADP) that proceeds further into glycolysis. This is advantageous, as it directs dihydroxyacetone phosphate down the same pathway as glyceraldehyde 3-phosphate, simplifying regulation.

Pay-off phase

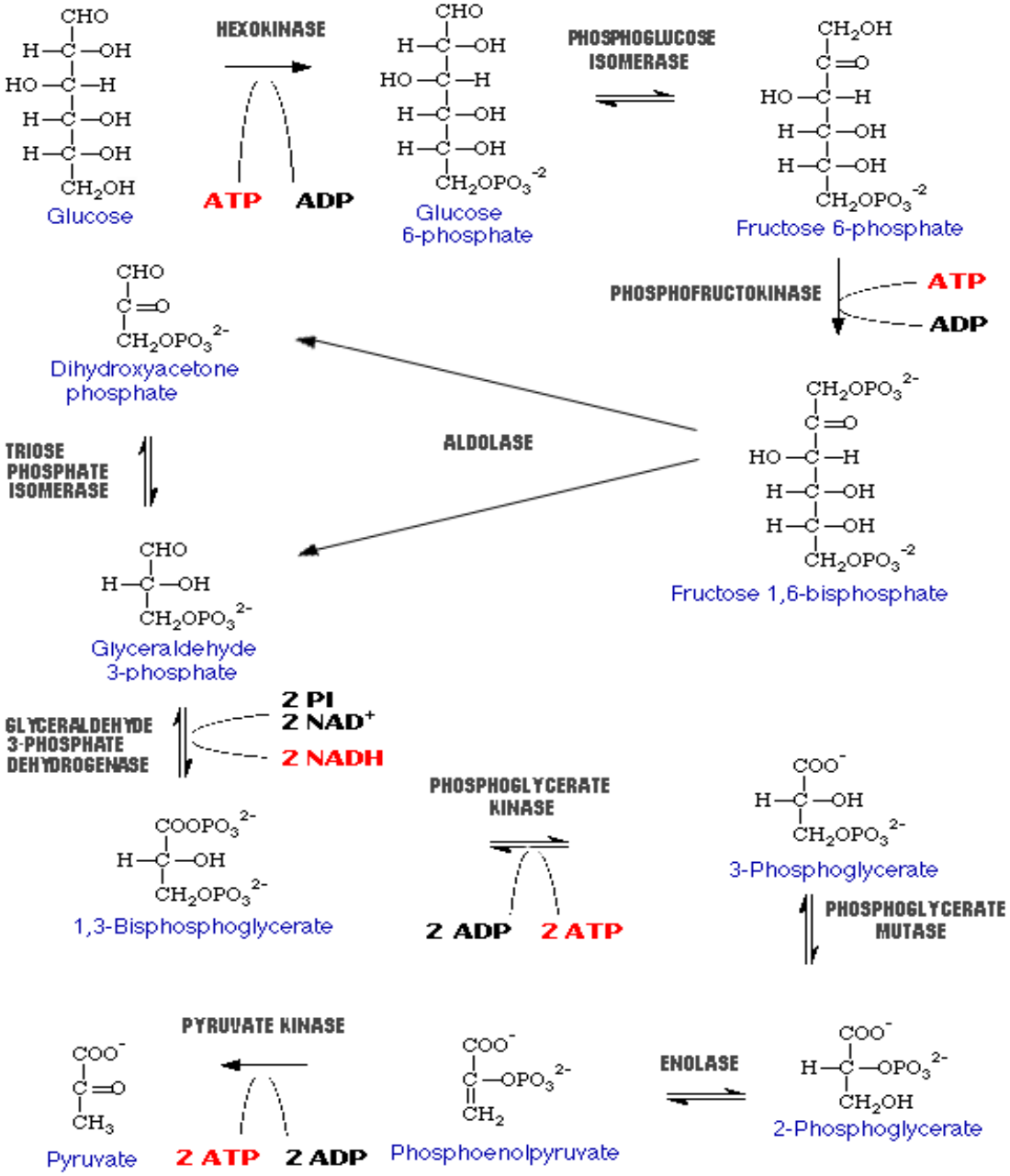
The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose. The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1, 3-bisphosphoglycerate. The hydrogen is used to reduce two molecules of NAD+, a hydrogen carrier, to give NADH + H^+ for each triose. Hydrogen atom balance and charge balance are both maintained because the phosphate (Pi) group actually exists in the form of a hydrogen phosphate anion (HPO42-), which dissociates to contribute the extra H+ ion and gives a net charge of -3 on both sides.

This step is the enzymatic transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP by phosphoglycerate kinase, forming ATP and 3-phosphoglycerate. At this step, glycolysis has reached the break-even point: 2 molecules of ATP were consumed, and 2 new molecules have now been synthesized. This step, one of the two substrate-level phosphorylation steps, requires ADP; thus, when the cell has plenty of ATP (and little ADP), this reaction does not occur. Because ATP decays relatively quickly when it is not metabolized, this is an important regulatory point in the glycolytic pathway. ADP actually exists as ADPMg-, and ATP as $ATPMg^2$-, balancing the charges at -5 both sides. A final substrate-level phosphorylation now forms a molecule of pyruvate and a molecule of ATP by means of the enzyme pyruvate kinase. This serves as an additional regulatory step, similar to the phosphoglycerate kinase step.

Regulation

Glycolysis is regulated by slowing down or speeding up certain steps in the glycolysis pathway. This is accomplished by inhibiting or activating the enzymes that are involved. The steps that are regulated may be determined by calculating the change in free energy, G, for each step. If a step's products and reactants are in equilibrium, then the step is assumed not to be regulated. Since the change in free energy is zero for a system at equilibrium, any step with a free energy change near zero is not being regulated. If a step is being regulated, then that step's enzyme is not converting reactants into products as fast as it could, resulting in a build-up of reactants, which would be converted to products if the enzyme were operating faster. Since the

reaction is thermodynamically favorable, the change in free energy for the step will be negative. A step with a large negative change in free energy is assumed to be regulated.



Post-glycolysis processes

The overall process of glycolysis is:

$$\text{Glucose} + 2\ NAD^+ + 2\ ADP + 2\ Pi \rightarrow 2\ \text{Pyruvate} + 2\ NADH + 2\ H^+ + 2\ ATP + 2\ H_2O$$

If glycolysis were to continue indefinitely, all of the NAD+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD+. How this is performed depends on which external electron acceptor is available.

Anoxic regeneration of NAD^+

One method of doing this is to simply have the pyruvate do the oxidation; in this process, pyruvate is converted to lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation:

$$\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + NAD^+$$

This process occurs in the bacteria involved in making yogurt (the lactic acid causes the milk to curdle). This process also occurs in animals under hypoxic (or partially anaerobic) conditions, found, for example, in overworked muscles that are starved of oxygen, or in infarcted heart muscle cells. In many tissues, this is a cellular last resort for energy; most animal tissue cannot tolerate anaerobic conditions for an extended period of time. Some organisms, such as yeast, convert NADH back to NAD+ in a process called ethanol fermentation. In this process, the pyruvate is converted first to acetaldehyde and carbon dioxide, then to ethanol.

Lactic acid fermentation and ethanol fermentation can occur in the absence of oxygen. This anaerobic fermentation allows many single-cell organisms to use glycolysis as their only energy source. Anoxic regeneration of NADH is only an effective means of energy production during short, intense exercise, providing energy for a period ranging from 10 seconds to 2 minutes and is dominant from about 10–30 seconds during a maximal effort. It replenishes very quickly over this period and produces 2 ATP molecules per glucose molecule, or about 5% of glucose's energy potential (38 ATP molecules in bacteria). The speed at which ATP is produced is about 100 times that of oxidative phosphorylation. The pH in the cytoplasm quickly drops when hydrogen ions accumulate in the muscle, eventually inhibiting enzymes involved in glycolysis.

The burning sensation in muscles during hard exercise can be attributed to the production of hydrogen ions during a shift to lactic acid fermentation as oxygen is converted to carbon dioxide by aerobic respiration faster than the body can replenish it. These hydrogen ions form a part of lactic acid along with lactate. The body falls back on this less efficient but faster method of producing ATP under low oxygen conditions. This is thought to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere and thus would represent a more ancient form of energy production in cells. The

liver later gets rid of this excess lactate by transforming it back into an important glycolytic intermediate called pyruvate; see Cori cycle. Fermenation of pyruvate to lactate is sometimes also called "anaerobic glycolysis", however, glycolysis ends with the production of pyruvate regardless in the presence or absence of oxygen.

Anaerobic respiration

In the above two examples of fermentation, NADH is oxidized by transferring two electrons to pyruvate. However, anaerobic bacteria use a wide variety of compounds as the terminal electron acceptors in cellular respiration: nitrogenous compounds, such as nitrates and nitrites; sulfur compounds, such as sulfates, sulfites, sulfur dioxide, and elemental sulfur; carbon dioxide; iron compounds; manganese compounds; cobalt compounds; and uranium compounds.

Aerobic respiration

In aerobic organisms, a complex mechanism has been developed to use the oxygen in air as the final electron acceptor.

- First, pyruvate is converted to acetyl-CoA and CO2 within the mitochondria in a process called pyruvate decarboxylation.
- Second, the acetyl-CoA enters the citric acid cycle, also known as Krebs Cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a hydrogen ion gradient across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce about 2.5 ATP for every NADH oxidized in a process called oxidative phosphorylation.

Intermediates for other pathways

This article concentrates on the catabolic role of glycolysis with regard to converting potential chemical energy to usable chemical energy during the oxidation of glucose to pyruvate. Many of the metabolites in the glycolytic pathway are also used by anabolic pathways, and, as a consequence, flux through the pathway is critical to maintain a supply of carbon skeletons for biosynthesis. In addition, not all carbon entering the pathway leaves as pyruvate and may be extracted at earlier stages to provide carbon compounds for other pathways. These metabolic pathways are all strongly reliant on glycolysis as a source of metabolites: and many more.

- Gluconeogenesis

- Lipid metabolism
- Pentose phosphate pathway
- Citric acid cycle, which in turn leads to:
- Amino acid synthesis
- Nucleotide synthesis
- Tetrapyrrole synthesis

From an anabolic metabolism perspective, the NADH has a role to drive synthetic reactions, doing so by directly or indirectly reducing the pool of NADP+ in the cell to NADPH, which is another important reducing agent for biosynthetic pathways in a cell.

Glycolysis in disease

Genetic diseases

Glycolytic mutations are generally rare due to importance of the metabolic pathway, this means that the majority of occurring mutations result in an inability for the cell to respire and therefore cause the death of the cell at an early stage. However, some mutations are seen with one notable example being Pyruvate kinase deficiency, leading to chronic hemolytic anemia.

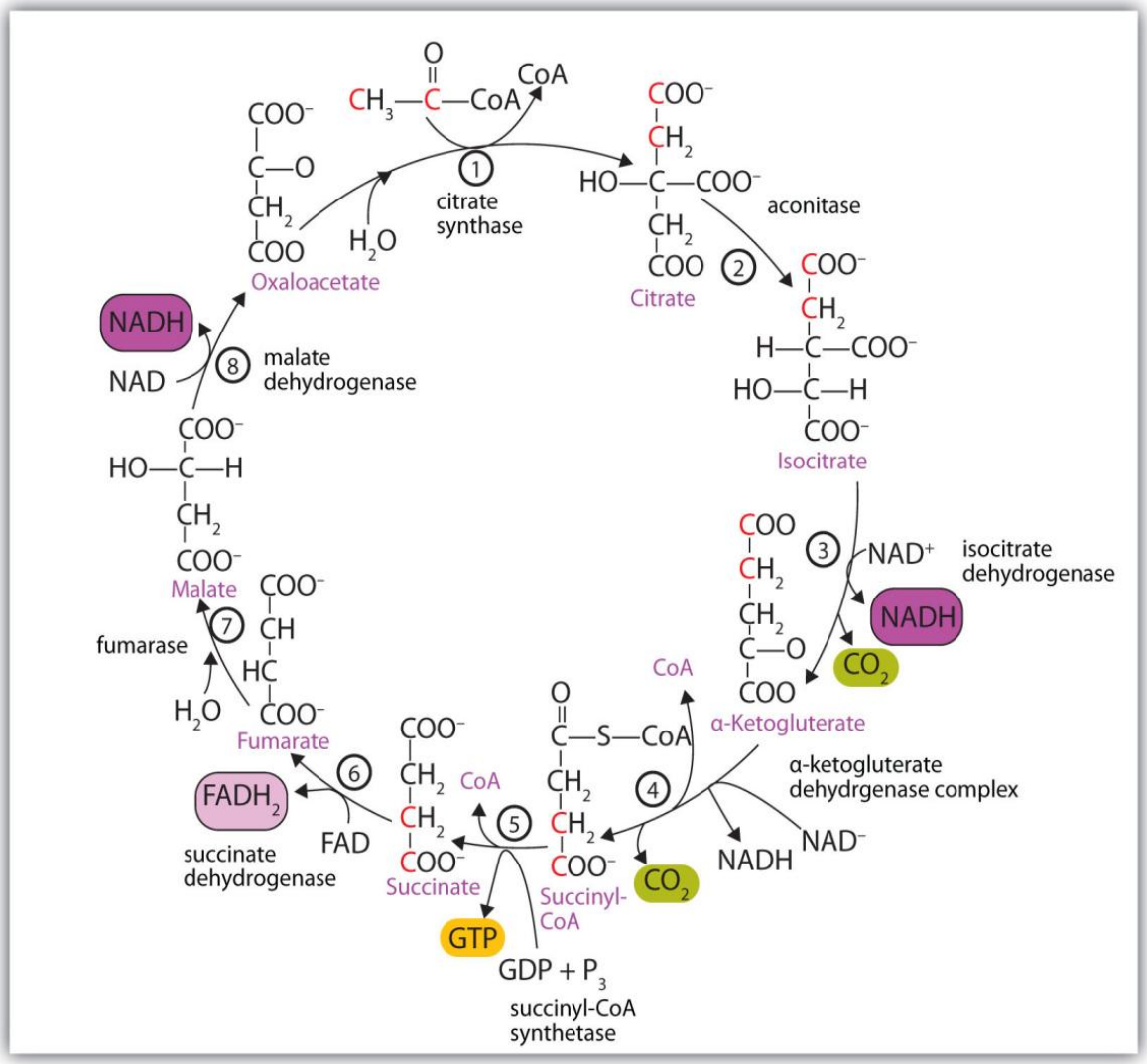
Cancer

Malignant rapidly growing tumor cells typically have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin. This phenomenon was first described in 1930 by Otto Warburg and is referred to as the Warburg effect. The Warburg hypothesis claims that cancer is primarily caused by dysfunctionality in mitochondrial metabolism, rather than because of uncontrolled growth of cells. A number of theories have been advanced to explain the Warburg effect. One such theory suggests that the increased glycolysis is a normal protective process of the body and that malignant change could be primarily caused by energy metabolism. This high glycolysis rate has important medical applications, as high aerobic glycolysis by malignant tumors is utilized clinically to diagnose and monitor treatment responses of cancers by imaging uptake of 2-18F-2-deoxyglucose (FDG) (a radioactive modified hexokinase substrate) with positron emission tomography (PET). There is ongoing research to affect mitochondrial metabolism and treat cancer by reducing glycolysis and thus starving cancerous cells in various new ways, including a ketogenic diet.

CITRIC ACID CYCLE

Tricarboxylic acid cycle, (TCA cycle), also called Krebs cycle and citric acid cycle, the second stage of cellular respiration, the three-stage process by which living cells break down organic fuel molecules in the presence of oxygen to harvest the energy they need to grow and divide. This metabolic process occurs in most plants, animals, fungi, and many bacteria. In all organisms except bacteria the TCA cycle is carried out in the matrix of intracellular structures called mitochondria. The TCA cycle plays a central role in the breakdown, or catabolism, of organic fuel molecules—i.e., glucose and some other sugars, fatty acids, and some amino acids. Before these rather large molecules can enter the TCA cycle they must be degraded into a two-carbon compound called acetyl coenzyme A (acetyl CoA). Once fed into the TCA cycle, acetyl CoA is converted into carbon dioxide and energy.

The TCA cycle consists of eight steps catalyzed by eight different enzymes . The cycle is initiated (1) when acetyl CoA reacts with the compound oxaloacetate to form citrate and to release coenzyme A (CoA-SH). Then, in a succession of reactions, (2) citrate is rearranged to form isocitrate; (3) isocitrate loses a molecule of carbon dioxide and then undergoes oxidation to form alpha-ketoglutarate; (4) alpha-ketoglutarate loses a molecule of carbon dioxide and is oxidized to form succinyl CoA; (5) succinyl CoA is enzymatically converted to succinate; (6) succinate is oxidized to fumarate; (7) fumarate is hydrated to produce malate; and, to end the cycle, (8) malate is oxidized to oxaloacetate. Each complete turn of the cycle results in the regeneration of oxaloacetate and the formation of two molecules of carbon dioxide.



Energy is produced in a number of steps in this cycle of reactions. In step 5, one molecule of adenosine triphosphate (ATP), the molecule that powers most cellular functions, is produced. Most of the energy obtained from the TCA cycle, however, is captured by the compounds nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) and converted later to ATP. Energy transfers occur through the relay of electrons from one substance to another, a process carried out through the chemical reactions known as oxidation and reduction, or redox reactions. (Oxidation involves the loss of electrons from a substance and reduction the addition of electrons.) For each turn of the TCA cycle, three molecules of NAD^+ are reduced to NADH and one molecule of FAD is reduced to $FADH_2$. These molecules then transfer their energy to the electron transport chain, a pathway that is part of the third stage of cellular

respiration. The electron transport chain in turn releases energy so that it can be converted to ATP through the process of oxidative phosphorylation.

Steps

Two carbon atoms are oxidized to CO_2, the energy from these reactions being transferred to other metabolic processes by GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the TCA cycle may later donate its electrons in oxidative phosphorylation to drive ATP synthesis; $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme functioning both in the TCA cycle and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$, therefore, facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the Succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

Major metabolic pathways converging on the TCA cycle

Several catabolic pathways converge on the TCA cycle. Reactions that form intermediates of the TCA cycle in order to replenish them (especially during the scarcity of the intermediates) are called anaplerotic reactions. The citric acid cycle is the third step in carbohydrate catabolism (the breakdown of sugars). Glycolysis breaks glucose (a six-carbon-molecule) down into pyruvate (a three-carbon molecule). In eukaryotes, pyruvate moves into the mitochondria. It is converted into acetyl-CoA by decarboxylation and enters the citric acid cycle.

In protein catabolism, proteins are broken down by proteases into their constituent amino acids. The carbon backbone of these amino acids can become a source of energy by being converted to acetyl-CoA and entering into the citric acid cycle. In fat catabolism, triglycerides are hydrolyzed to break them into fatty acids and glycerol. In the liver the glycerol can be converted into glucose via dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by way of gluconeogenesis. In many tissues, especially heart tissue, fatty acids are broken down through a process known as beta oxidation, which results in acetyl-CoA, which can be used in the citric acid cycle. Beta oxidation of fatty acids with an odd number of methylene bridges produces propionyl CoA, which is then converted into succinyl-CoA and fed into the citric acid cycle. The total energy gained from the complete breakdown of one molecule of glucose by glycolysis, the citric acid cycle, and oxidative phosphorylation equals about 30 ATP molecules, in eukaryotes. The citric acid cycle is called an amphibolic pathway because it participates in both catabolism and anabolism.

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DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **METABOLIC PATHWAYS**
SEMESTER : **V**
SUBJECT CODE : **15BCU501** **CLASS** : **III B.Sc.BC**

UNIT II-- COURSE MATERIAL

Carbohydrate metabolism

Introduction, fate of absorbed of carbohydrate, utilization of glucose, general processes of carbohydrate metabolism-glycolysis and citric acid cycle, glycogenesis, glycogenolysis and gluconeogenesis. Alternate pathways of carbohydrate metabolism-pentose phosphate pathway (HMP shunt), Glucuronic acid cycle and Glyoxylate cycle

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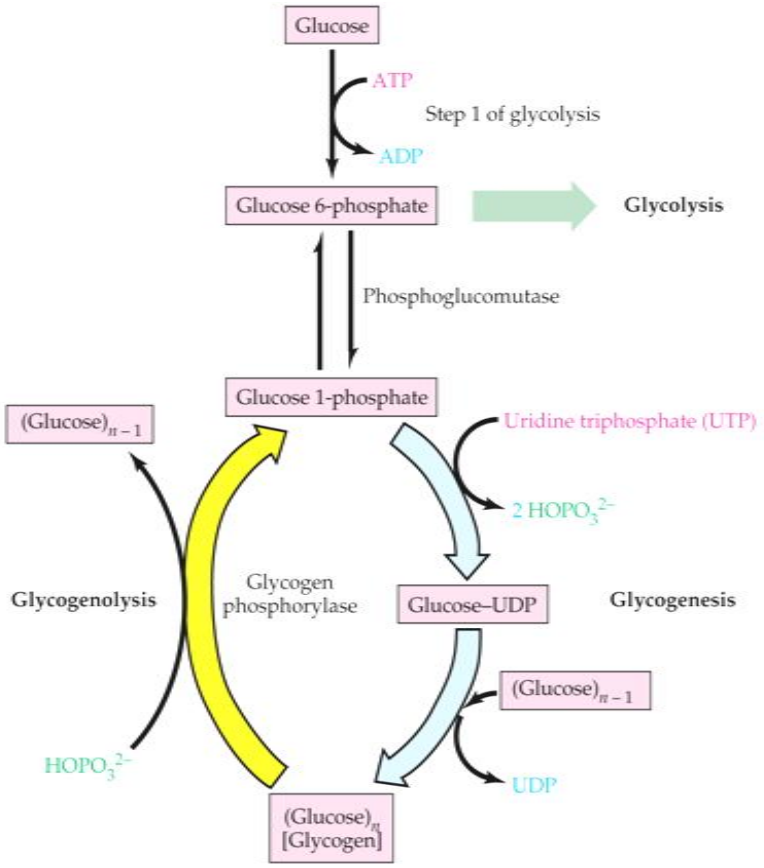
UNIT-II: CARBOHYDRATE METABOLISM

GLYCOGENESIS

Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage. This process is activated during rest periods following the Cori cycle, in the liver, and also activated by insulin in response to high glucose levels, for example after a carbohydrate-containing meal.

Steps

Glucose is converted into glucose-6-phosphate by the action of glucokinase or Hexokinase. Glucose-6-phosphate is converted into glucose-1-phosphate by the action of Phosphoglucomutase, passing through an obligatory intermediate step of glucose-1,6-bisphosphate. Glucose-1-phosphate is converted into UDP-glucose by the action of Uridyl Transferase (also called UDP-glucose pyrophosphorylase) and pyrophosphate is formed, which is hydrolysed by pyrophosphatase into 2 molecules of Pi.



Glucose molecules are assembled in a chain by glycogen synthase, which must act on a pre-existing glycogen primer or glycogenin (small protein that forms the primer). The mechanism for joining glucose units is that glycogen synthase binds to UDPG, causing it to break down into an oxonium ion, also formed in glycogenolysis. This oxonium ion can readily add to the 4-hydroxyl group of a glucosyl residue on the 4 end of the glycogen chain.

Control and regulations

Glycogenesis responds to hormonal control.

One of the main forms of control is the varied phosphorylation of glycogen synthase and glycogen phosphorylase. This is regulated by enzymes under the control of hormonal activity, which is in turn regulated by many factors. As such, there are many different possible effectors when compared to allosteric systems of regulation.

Epinephrine

Glycogen phosphorylase is activated by phosphorylation, whereas glycogen synthase is inhibited. Glycogen phosphorylase is converted from its less active "b" form to an active "a" form by the enzyme phosphorylase kinase. This latter enzyme is itself activated by protein kinase A and deactivated by phosphoprotein phosphatase-1.

Protein kinase A itself is activated by the hormone adrenaline. Epinephrine binds to a receptor protein that activates adenylate cyclase. The latter enzyme causes the formation of cyclic AMP from ATP; two molecules of cyclic AMP bind to the regulatory subunit of protein kinase A, which activates it allowing the catalytic subunit of protein kinase A to dissociate from the assembly and to phosphorylate other proteins. Returning to glycogen phosphorylase, the less active "b" form can itself be activated without the conformational change. 5'AMP acts as an allosteric activator, whereas ATP is an inhibitor, as already seen with phosphofructokinase control, helping to change the rate of flux in response to energy demand.

Epinephrine not only activates glycogen phosphorylase but also inhibits glycogen synthase. This amplifies the effect of activating glycogen phosphorylase. This inhibition is achieved by a similar mechanism, as protein kinase A acts to phosphorylate the enzyme, which lowers activity. This is known as co-ordinate reciprocal control. Refer to glycolysis for further information of the regulation of glycogenesis.

Insulin

Insulin has an antagonistic effect to epinephrine signaling via the beta-adrenergic receptor (G-Protein coupled receptor). When insulin binds to its receptor (insulin receptor), it results in the activation (phosphorylation) of Akt which in turn activates Phosphodiesterase (PDE). PDE then will inhibit cyclic AMP (cAMP) action and cause inactivation of PKA which will cause Hormone Sensitive Lipase (HSL) to be dephosphorylated and inactive so that lipolysis and lipogenesis is not occurring simultaneously.

Calcium ions

Calcium ions or cyclic AMP (cAMP) act as secondary messengers. This is an example of negative control. The calcium ions activate phosphorylase kinase. This activates glycogen phosphorylase and inhibits glycogen synthase.

GLYCOGENOLYSIS

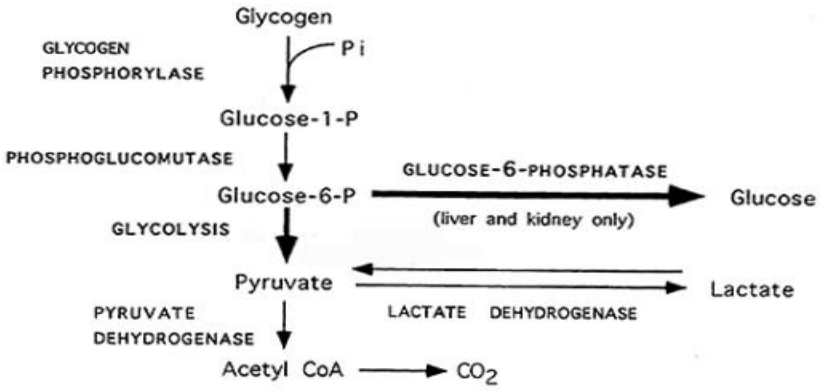
Glycogenolysis is the breakdown of glycogen (n) to glucose-1-phosphate and glycogen (n-1). Glycogen branches are catabolized by the sequential removal of glucose monomers via phosphorolysis, by the enzyme glycogen phosphorylase

Mechanism

The overall reaction for the breakdown of glycogen to glucose-1-phosphate is:

Glycogen (n residues) + Pi is in equilibrium with glycogen(n-1 residues) + glucose-1-phosphate

Here, **glycogen phosphorylase** cleaves the bond linking a terminal glucose residue to a glycogen branch by substitution of a phosphoryl group for the linkage. Glucose-1-phosphate is converted to glucose-6-phosphate by the enzyme phosphoglucomutase. Glucose residues are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with a linkage. Glycogen **debranching enzyme** then transfers three of the remaining four glucose units to the end of another glycogen branch. This exposes the branching point, which is hydrolysed by glucosidase, removing the final glucose residue of the branch as a molecule of glucose and eliminating the branch. This is the only case in which a glycogen metabolite is not glucose-1-phosphate. The glucose is subsequently phosphorylated to glucose-6-phosphate by hexokinase.



Function

Glycogenolysis takes place in the cells of the muscle and liver tissues in response to hormonal and neural signals. In particular, glycogenolysis plays an important role in the fight-or-flight response and the regulation of glucose levels in the blood. In myocytes (muscle cells), glycogen degradation serves to provide an immediate source of glucose-6-phosphate for glycolysis, to provide energy for muscle contraction.

In hepatocytes (liver cells), the main purpose of the breakdown of glycogen is for the release of glucose into the bloodstream for uptake by other cells. The phosphate group of glucose-6-phosphate is removed by the enzyme glucose-6-phosphatase, which is not present in myocytes, and the free glucose exits the cell via GLUT2 facilitated diffusion channels in the hepatocyte cell membrane.

Regulation

Glycogenolysis is regulated hormonally in response to blood sugar levels by glucagon and insulin, and stimulated by epinephrine during the fight-or-flight response. In myocytes, glycogen degradation may also be stimulated by neural signals.

Clinical significance

Parenteral (intravenous) administration of glucagon is a common human medical intervention in diabetic emergencies when sugar cannot be given orally. It can also be administered intramuscularly.

GLUCONEOGENESIS

Gluconeogenesis is the biosynthesis of new glucose, (i.e. not glucose from glycogen). This process is frequently referred to as endogenous glucose production (EGP). The production of glucose from other carbon skeletons is necessary since the testes, erythrocytes and kidney

medulla exclusively utilize glucose for ATP production. The brain also utilizes large amounts of the daily glucose consumed or produced via gluconeogenesis. However, in addition to glucose, the brain can derive energy from ketone bodies which are converted to acetyl-CoA and shunted into the TCA cycle. The primary carbon skeletons used for gluconeogenesis are derived from pyruvate, lactate, glycerol, and the amino acids alanine and glutamine. The liver is the major site of gluconeogenesis, however, as discussed below, the kidney and the small intestine also have important roles to play in this pathway. Synthesis of glucose from three and four carbon precursors is essentially a reversal of glycolysis.

Precursor

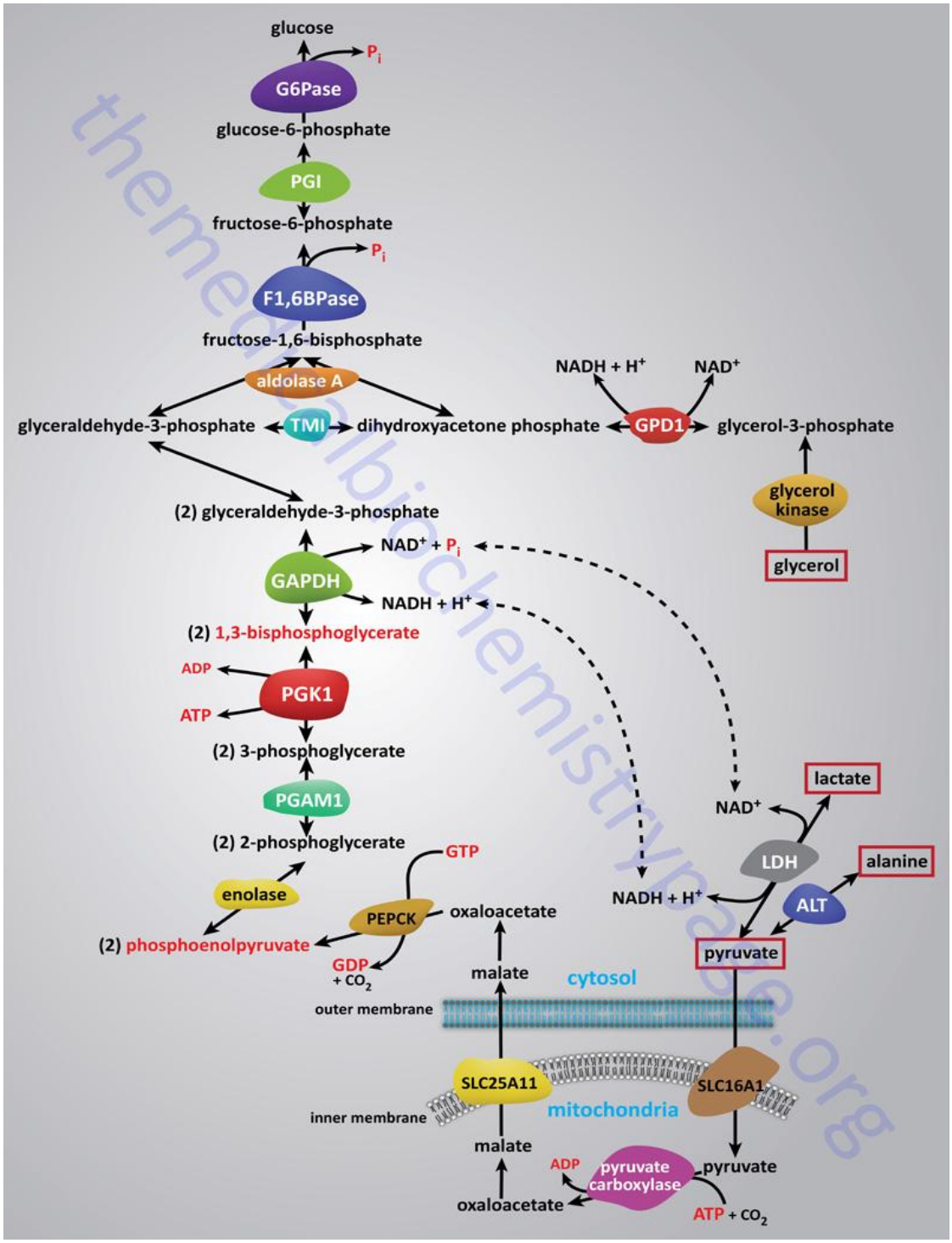
In humans the main gluconeogenic precursors are lactate, glycerol (which is a part of the triacylglycerol molecule), alanine and glutamine. Altogether, they account for over 90% of the overall gluconeogenesis. Other glucogenic amino acid as well as all citric acid cycle intermediates, the latter through conversion to oxaloacetate, can also function as substrates for gluconeogenesis. In ruminants, propionate is the principal gluconeogenic substrate.

Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. Transamination or deamination of amino acids facilitates entering of their carbon skeleton into the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

Whether even-chain fatty acids can be converted into glucose in animals has been a longstanding question in biochemistry. It is known that odd-chain fatty acids can be oxidized to yield propionyl CoA, a precursor for succinyl CoA, which can be converted to pyruvate and enter into gluconeogenesis. In plants, specifically seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis.

The existence of glyoxylate cycles in humans has not been established, and it is widely held that fatty acids cannot be converted to glucose in humans directly. However, carbon-14 has been shown to end up in glucose when it is supplied in fatty acids. Despite these findings, it is considered unlikely that the 2-carbon acetyl-CoA derived from the oxidation of fatty acids would produce a net yield of glucose via the citric acid cycle - however, acetyl-CoA can be converted into pyruvate and lactate through the ketogenic pathway.Put simply, acetic acid (in the form of

acetyl-CoA) is used to partially produce glucose; acetyl groups can only form part of the glucose molecules (not the 5th carbon atom) and require extra substrates (such as pyruvate) in order to form the rest of the glucose molecule. But a roundabout pathway does lead from acetyl-coA to pyruvate, via acetoacetate, acetone, acetol and then either propylene glycol or methylglyoxal.



Location

In mammals, gluconeogenesis is restricted to the liver, the kidney and possibly the intestine. However these organs use somewhat different gluconeogenic precursors. The liver uses primarily lactate, alanine and glycerol while the kidney uses lactate, glutamine and glycerol. Propionate is the principal substrate for gluconeogenesis in the ruminant liver, and the ruminant liver may make increased use of gluconeogenic amino acids, e.g. alanine, when glucose demand is increased. The capacity of liver cells to use lactate for gluconeogenesis declines from the preruminant stage to the ruminant stage in calves and lambs. In sheep kidney tissue, very high rates of gluconeogenesis from propionate have been observed. The intestine uses mostly glutamine and glycerol.

In all species, the formation of oxaloacetate from pyruvate and TCA cycle intermediates is restricted to the mitochondrion, and the enzymes that convert Phosphoenolpyruvic acid (PEP) to glucose are found in the cytosol. The location of the enzyme that links these two parts of gluconeogenesis by converting oxaloacetate to PEP, PEP carboxykinase, is variable by species: it can be found entirely within the mitochondria, entirely within the cytosol, or dispersed evenly between the two, as it is in humans. Transport of PEP across the mitochondrial membrane is accomplished by dedicated transport proteins; however no such proteins exist for oxaloacetate. Therefore, in species that lack intra-mitochondrial PEP carboxykinase, oxaloacetate must be converted into malate or aspartate, exported from the mitochondrion, and converted back into oxaloacetate in order to allow gluconeogenesis to continue.

Pathway

Gluconeogenesis is a pathway consisting of a series of eleven enzyme-catalyzed reactions. The pathway may begin in the mitochondria or cytoplasm, this being dependent on the substrate being used. Many of the reactions are the reversible steps found in glycolysis. Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate by the carboxylation of pyruvate. This reaction also requires one molecule of ATP, and is catalyzed by pyruvate carboxylase. This enzyme is stimulated by high levels of acetyl-CoA (produced in -oxidation in the liver) and inhibited by high levels of ADP and glucose.

Oxaloacetate is reduced to malate using NADH, a step required for its transportation out of the mitochondria. Malate is oxidized to oxaloacetate using NAD^+ in the cytosol, where the remaining steps of gluconeogenesis take place. Oxaloacetate is decarboxylated and then

phosphorylated to form phosphoenolpyruvate using the enzyme phosphoenolpyruvate carboxykinase. A molecule of GTP is hydrolyzed to GDP during this reaction. The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose 6-phosphate, using one water molecule and releasing one phosphate. This is also the rate-limiting step of gluconeogenesis. Glucose-6-phosphate is formed from fructose 6-phosphate by phosphoglucoisomerase. Glucose-6-phosphate can be used in other metabolic pathways or dephosphorylated to free glucose. Whereas free glucose can easily diffuse in and out of the cell, the phosphorylated form (glucose-6-phosphate) is locked in the cell, a mechanism by which intracellular glucose levels are controlled by cells. The final reaction of gluconeogenesis, the formation of glucose, occurs in the lumen of the endoplasmic reticulum, where glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is shuttled into the cytoplasm by glucose transporters located in the endoplasmic reticulum's membrane.

ALTERNATIVE PATHWAYS OF GLUCOSE METABOLISM

PENTOSE PHOSPHATE PATHWAY (HMP SHUNT)

The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a biochemical pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars). While it does involve oxidation of glucose, its primary role is anabolic rather than catabolic. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non-oxidative synthesis of 5-carbon sugars. For most organisms, the pentose phosphate pathway takes place in the cytosol; in plants, most steps take place in plastids.

Similar to glycolysis, the pentose phosphate pathway appears to have a very ancient evolutionary origin. The reactions of this pathway are (mostly) enzyme catalysed in modern cells. They also occur however non-enzymatically under conditions that replicate those of the Archean ocean, and are then catalyzed by metal ions, ferrous iron Fe (II) in particular. The origins of the pathway could thus date back to the prebiotic world.

Pathway

The generation of reducing equivalents, in the form of NADPH, used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis). Production of ribose-5-phosphate (R5P), used in the synthesis of nucleotides and nucleic acids. Production of erythrose-4-

phosphate (E4P), used in the synthesis of aromatic amino acids. Aromatic amino acids, in turn, are precursors for many biosynthetic pathways, including the lignin in wood.

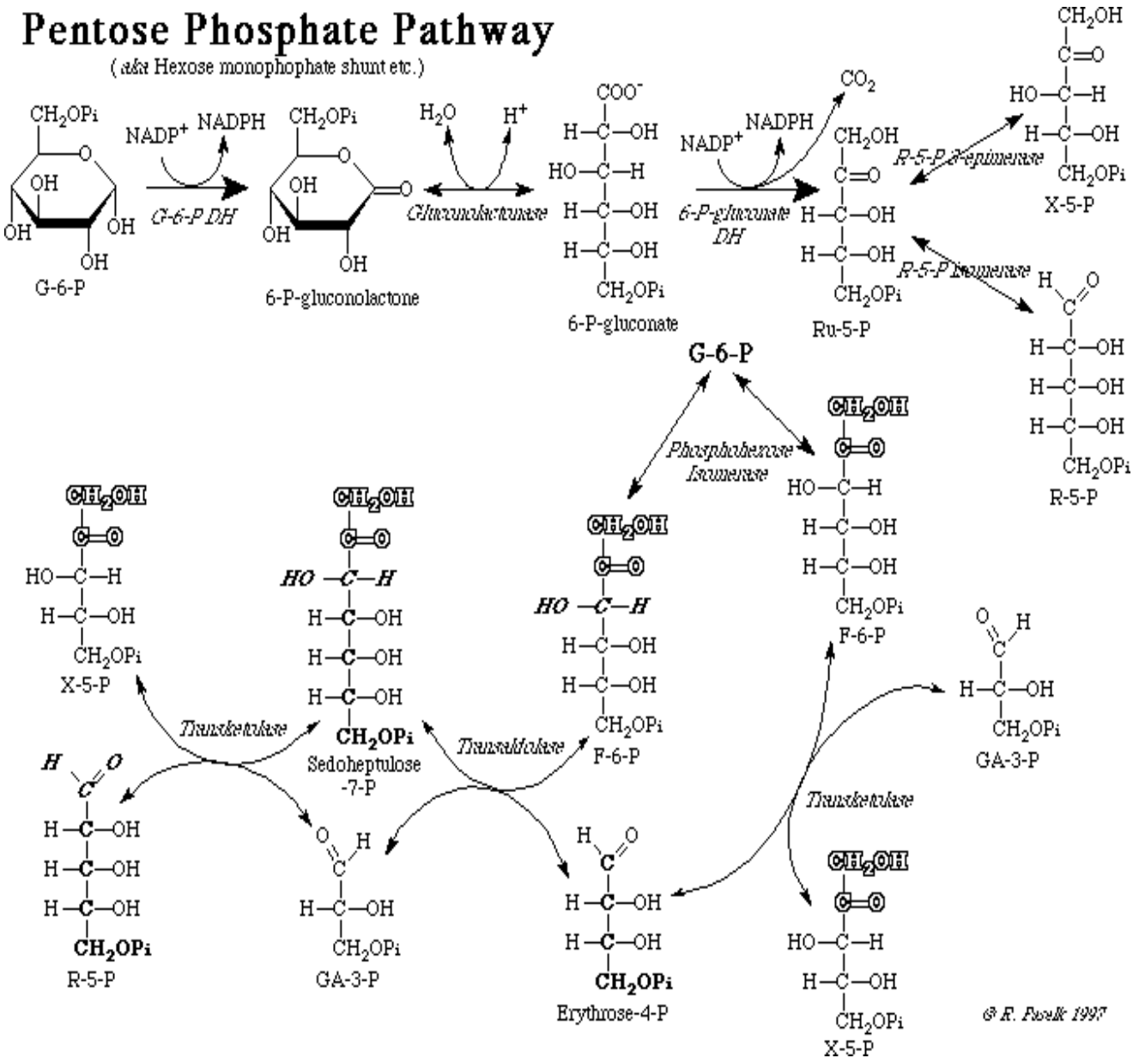
Dietary pentose sugars derived from the digestion of nucleic acids may be metabolized through the pentose phosphate pathway, and the carbon skeletons of dietary carbohydrates may be converted into glycolytic/gluconeogenic intermediates. In mammals, the PPP occurs exclusively in the cytoplasm, and is found to be most active in the liver, mammary gland and adrenal cortex in the human. The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans.

One of the uses of NADPH in the cell is to prevent oxidative stress. It reduces glutathione via glutathione reductase, which converts reactive H2O2 into H2O by glutathione peroxidase. If absent, the H_2O_2 would be converted to hydroxyl free radicals by Fenton chemistry, which can attack the cell. Erythrocytes, for example, generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.Hydrogen peroxide is also generated for phagocytes in a process often referred to as a respiratory burst.

Phases

Oxidative phase

In this phase, two molecules of NADP+ are reduced to NADPH, utilizing the energy from the conversion of glucose-6-phosphate into ribulose 5-phosphate.



Regulation

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme of this pathway. It is allosterically stimulated by $NADP^+$. The ratio of NADPH: $NADP^+$ is normally about 100:1 in liver cytosol[citation needed]. This makes the cytosol a highly-reducing environment. An NADPH-utilizing pathway forms $NADP^+$, which stimulates Glucose-6-phosphate ehydrogenase to produce more NADPH. This step is also inhibited by acetyl CoA.

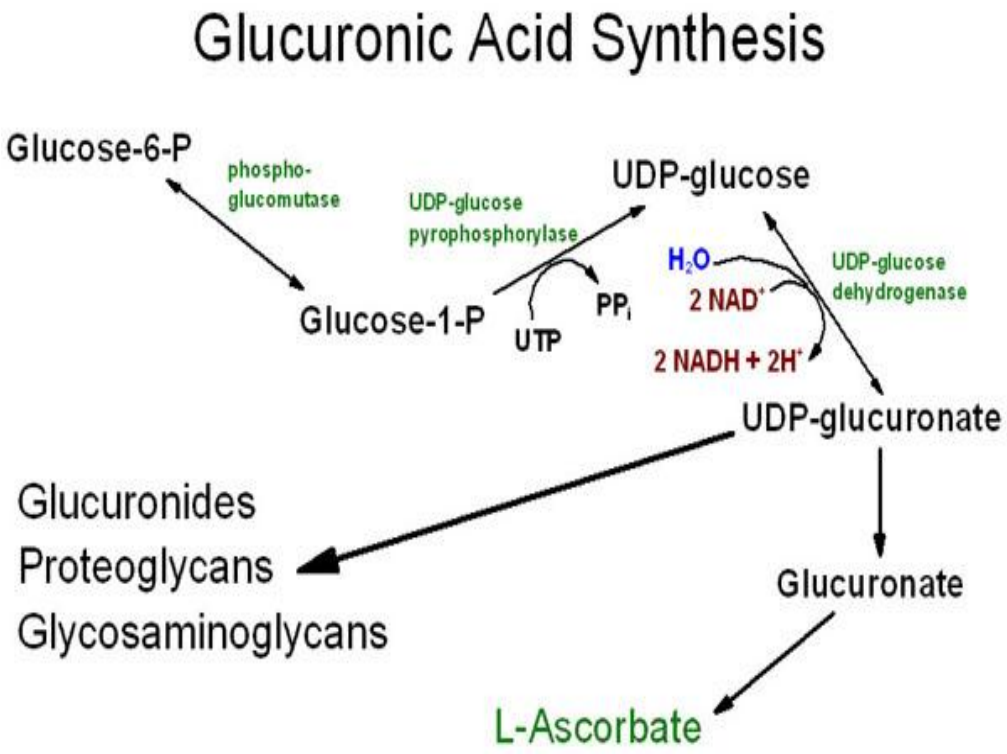
Erythrocytes and the pentose phosphate pathway

Several deficiencies in the level of activity of glucose-6-phosphate dehydrogenase have been observed to be associated with resistance to the malarial parasite Plasmodium falciparum among

individuals of Mediterranean and African descent. The basis for this resistance may be a weakening of the red cell membrane (the erythrocyte is the host cell for the parasite) such that it cannot sustain the parasitic life cycle long enough for productive growth.

GLUCURONIC ACID CYCLE

Glucuronic acid is a carboxylic acid. Its structure is similar to that of glucose. However, glucuronic acid's sixth carbon is oxidized to a carboxylic acid. Its formula is C_6H_{10}. Glucuronic acid should not be confused with gluconic acid, a linear carboxylic acid resulting from the oxidation of a different carbon of glucose.



Functions

Glucuronic acid is common in carbohydrate chains of proteoglycans. It is part of mucous animal secretions (such as saliva), cell glycocalyx, and intercellular matrix (for instance hyaluronan).

Use

- Determination of urinary steroids and of steroid conjugates in blood.
- Both glucuronic acid and gluconic acid are reported to be contained in some commercially available brands of Kombucha.

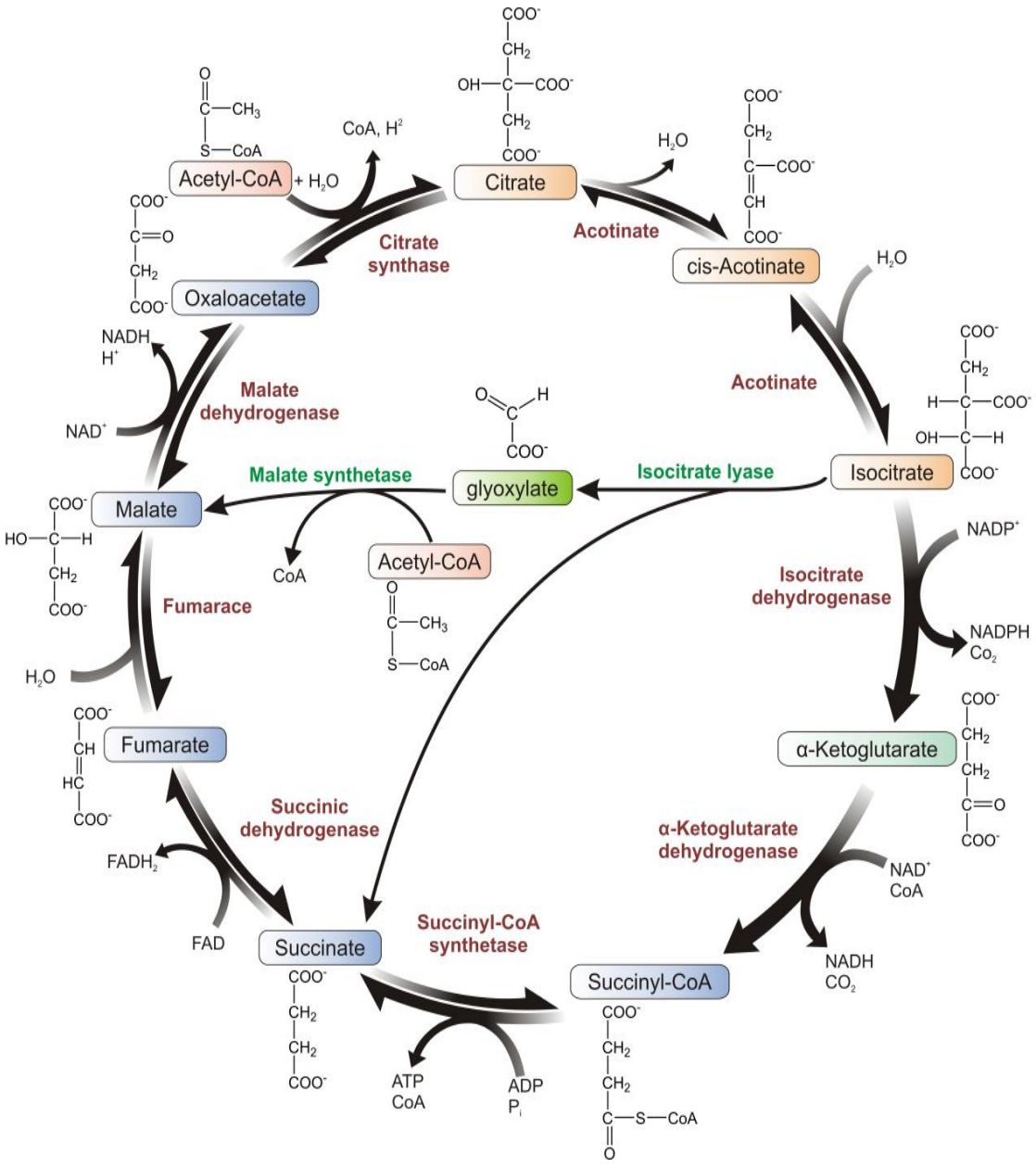
- In all plants and mammals-other than guinea pigs and primates-glucuronic acid is a precursor of ascorbic acid, also known as vitamin C.

Glucuronidases

Glucuronidases are those enzymes that hydrolyze the glycosidic bond between glucuronic acid and some other compound.

GLYOXYLATE CYCLE

The glyoxylate cycle, a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi. The glyoxylate cycle centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates. In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis. In recent years, however, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals and suggests that animals encode alternative enzymes of the cycle that differ in function from known MS and ICL in non-metazoan species.



Similarities with TCA cycle

The glyoxylate cycle utilizes three of the five enzymes associated with the tricarboxylic acid cycle and shares many of its intermediate steps. The two cycles vary when, in the glyoxylate cycle, ICL converts isocitrate into glyoxylate and succinate instead of -ketoglutarate as seen in the TCA cycle. This bypasses the decarboxylation steps that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose. The glyoxylate cycle then continues on, using glyoxylate and acetyl-CoA to produce malate.

Role in gluconeogenesis

Fatty acids from lipids are commonly used as an energy source by vertebrates as fatty acids are degraded through beta oxidation into acetate molecules. This acetate, bound to the active thiol group of coenzyme A, enters the citric acid cycle (TCA cycle) where it is fully oxidized to carbon dioxide. This pathway thus allows cells to obtain energy from fat. To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.

Cell-wall containing organisms, such as plants, fungi, and bacteria, require very large amounts of carbohydrates during growth for the biosynthesis of complex structural polysaccharides, such as cellulose, glucans, and chitin. In these organisms, in the absence of available carbohydrates (for example, in certain microbial environments or during seed germination in plants), the glyoxylate cycle permits the synthesis of glucose from lipids via acetate generated in fatty acid -oxidation.

The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO_2. The two initial steps of the glyoxylate cycle are identical to those in the citric acid cycle: acetate citrate isocitrate. In the next step, catalyzed by the first glyoxylate cycle enzyme, isocitrate lyase, isocitrate undergoes cleavage into succinate and glyoxylate. Glyoxylate condenses with acetyl-CoA, yielding malate. Both malate and oxaloacetate can be converted into phosphoenolpyruvate, which is the product of phosphoenolpyruvate carboxykinase, the first enzyme in gluconeogenesis. The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids. Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

Function in organisms

Plants

In plants the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. The seed cannot produce biomass using photosynthesis because of lack of an organ to perform this function. The lipid stores of germinating seeds are used for the formation of the carbohydrates that fuel the growth and development of the organism.

The glyoxylate cycle can also provide plants with another aspect of metabolic diversity. This cycle allows plants to take in acetate both as a carbon source and as a source of energy. Acetate

is converted to Acetyl CoA (similar to the TCA cycle). This Acetyl CoA can proceed through the glyoxylate cycle, and some succinate is released during the cycle. The four carbon succinate molecule can be transformed into a variety of carbohydrates through combinations of other metabolic processes; the plant can synthesize molecules using acetate as a source for carbon. The Acetyl CoA can also react with glyoxylate to produce some NADPH from $NADP^+$, which is used to drive energy synthesis in the form of ATP later in the Electron Transport Chain.

Pathogenic fungi

The glyoxylate cycle may serve an entirely different purpose in some species of pathogenic fungi. The levels of the main enzymes of the glyoxylate cycle, ICL and MS, are greatly increased upon contact with a human host. Mutants of a particular species of fungi that lacked ICL were also significantly less virulent in studies with mice compared to the wild type. The exact link between these two observations is still being explored, but it can be concluded that the glyoxylate cycle is a significant factor in the pathogenesis of these microbes.

Vertebrates

Vertebrates were once thought to be unable to perform this cycle because there was no evidence of its two key enzymes, isocitrate lyase and malate synthase. However, some research suggests that this pathway may exist in some, if not all, vertebrates. Specifically, some studies show evidence of components of the glyoxylate cycle existing in significant amounts in the liver tissue of chickens. Data such as these support the idea that the cycle could theoretically occur in even the most complex vertebrates. Other experiments have also provided evidence that the cycle is present among certain insect and marine invertebrate species, as well as strong evidence of the cycle's presence in nematode species. However, other experiments refute this claim Some publications conflict on the presence of the cycle in mammals: for example, one paper has stated that the glyoxalate cycle is active in hibernating bears, but this report was disputed in a later paper. On the other hand, no functional genes related to known forms of malate synthase or isocitrate lyase have been identified in placental mammal genomes, while malate synthase appears to be functional in some non-placental mammals and other vertebrates. Vitamin D may regulate this pathway in vertebrates.

S. No	Question	Option A	Option B	Option C	Option D	Answer
1.	Our body can get pentoses from	Glycolytic pathway	Uronic acid pathway	TCA cycle	HMP shunt	HMP shunt
2.	The number of molecules of ATP produced by the total oxidation of acetyl CoA in TCA cycle is	6	8	10	12	12
3.	Which of the following metabolite integrates glucose and fatty acid metabolism?	Acetyl CoA	Pyruvate	Citrate	Lactate	Acetyl CoA
4.	Gluconeogenesis is decreased by	Glucagon	Epinephrine	Glucocorticoids	Insulin	Insulin
5.	The formation of citrate from oxalo acetate and acetyl CoA is	Oxidation	Reduction	Condensation	Hydrolysis	Condensation
6.	The carrier of the citric acid cycle is	Succinate	Fumarate	Malate	Oxaloacetate	Oxaloacetate
7.	The Key enzymes in glycolysis are	Hexokinase	Hexokinase and Phosphofructokinase	Hexokinase, Phosphofructokinase and pyruvate kinase	Hexokinase, Phosphofructokinase and fructose -1-phosphatase	Hexokinase, Phosphofructokinase and pyruvate kinase
8.	The complete oxidation of glucose occurs in	Glycolysis	HMP shunt	Glycolysis and TCA cycle	TCA cycle	Glycolysis and TCA cycle
9.	TCA Cycle takes place in	Cytosol	Ribosomes	Mitochondria	Nucleus	Mitochondria
10.	TCA Cycle is called as amphibolic pathway because it	Produces energy	It is catabolic and anabolic	Produces CO_2 and H_2O	Occurs in mitochondria	It is catabolic and anabolic
11.	Von Gierke's disease is due to deficiency of enzyme	Glucose-6-phosphatase	Glucose -1-phosphatase	Fructose-6-phosphatase	Fructose-1-phosphatase	Glucose-6-phosphatase
12.	Pentose provided by HMP shunt is used for	Energy production	Fatty acid production	Nucleic acid synthesis	Steroid synthesis	Nucleic acid synthesis
13.	Essential pentosuria is due to deficiency of enzyme	Xylulose reductase	Xylitol dehydrogenase	Xylitol synthetase	Xylitol decarboxylase	Xylitol dehydrogenase

14.	Rate limiting enzyme in glycogenolysis is	Phosphorylase	Phosphoglucomutase	Glucose 6 phosphatase	Fructose 1,6 phosphatase	Phosphorylase
15.	Rate limiting enzyme in glycogenesis is	Glucokinase	Phophoglucomutase	UDPG phosphorylase	Glycogen synthetase	Glycogen synthetase
16.	Regeneration of ____is required for continues Tricarboxylic acid cycle	Pyruvic acid	Oxaloacetic acid	α-oxoglutaric acid	Malic acid	Oxaloacetic acid
17.	Which one of the following is a rate limiting enzyme of gluconeogenesis?	Hexokinase	Phsophofructokinase	Pyruvate enol pyruvate carboxy kinase	Pyruvate kinase	Pyruvate enol pyruvate carboxy kinase
18.	Different forms of same enzymes that catalyze one particular reaction are	isoenzymes	coenzymes	abzymes	Synzyme	isoenzymes
19.	Gluconeogenesis occurs in	Mitochondria	Cytosol	Mitochondria and Cytosol	glyoxysome	Mitochondria and Cytosol
20.	Regulation of gluconeogenesis reciprocal to that of	Glycogenesis	Glycogenolysis	Glycolysis	HMP Shunt	Glycolysis
21.	During glycolysis, a net gain of four ATPs are generated by what process?	Chemiosmosis	ADP processing	Substrate level phosphorylation	Electron transport chain	Substrate level phosphorylation
22.	Where does the TCA cycle take place ?	Mitochondrial matrix	Cytoplasm	Mitochondrial membrane	Cytoplasmic membrane	Mitochondrial matrix
23.	In aerobic respiration when is the first molecule of carbon dioxide released?	During chemiosmosis	When pyruvic acid is reduced to lactic acid	During the conversion step when pyruvic acid is converted to acetyl-CoA	When glucose is phosphorylated in glycolysis	During the conversion step when pyruvic acid is converted to acetyl-CoA
24.	What pathway is a significant intermediate source of pentoses for nucleic acid synthesis?	Glycolysis	TCA cycle	Electron transport chain	Hexose monophosphate shunt	Hexose monophosphate shunt
25.	One turn of the citric acid cycle produces	2 NADH, 2 $FADH_2$, 2 ATP	3 NADH, 1 $FADH_2$, 1 ATP	3 NADH, 2 $FADH_2$, 1 ATP	3 NADH, 1 $FADH_2$, 2 ATP	3 NADH, 1 $FADH_2$, 1 ATP

26.	**The preparatory reaction breaks**	Glucose into pyruvates	Pyruvates into glucose	Pyruvates into acetyl-coA and carbon dioxide	Pyruvates into acetyl-coa and water	Pyruvates into acetyl-coA and carbon dioxide
27.	**An enzymatic reaction that is most similar to the conversion of pyruvate to acetyl CoA is catalyzed by**	Citrate synthase	Alpha-ketoglutarate dehydrogenase	Succinyl-coa synthetase	Isocitrate dehydrogenase	Alpha-ketoglutarate dehydrogenase
28.	**Intermediates of the citric acid cycle are replenished by a reaction converting pyruvate to**	Oxaloacetate	Citrate	Alpha-ketoglutarate	Succinyl-coA	Oxaloacetate
29.	The correct sequence for aerobic metabolic breakdown of glucose is	Glycolysis–preparatory reaction–cirtric acid cycle–electron transport system	Preparatory reaction–glycolysis–electron transport–citric acid cycle	Electron transport system–citric acid cycle–preparatory reaction–glycolysis	Glycolysis–citric acid cycle–electron transport system–preparatory reaction	Glycolysis–preparatory reaction–cirtric acid cycle–electron transport system
30.	End product of TCA cycle is	Citric acid	Pyruvic acid	Lactic acid	CO_2 and water	CO_2 and water
31.	Embden-Meyerhof pathway referred as	Gluconeogenesis	Glycolysis	Citric acid	Glycogenesis	Glycolysis
32.	_____ is the precursor for the synthesis of ascorbic acid	L-gulonate	L-alanine	Methionine	L-aspartic acid	L-gulonate
33.	Enzymes involved with glyoxylate cycle is	Isocitrate hydrogenase and Isocitrate	Isocitrate dehydrogenase and malate synthetase	Isocitrate lyase and malate synthetase	citrate synthetase and malate synthetase	Isocitrate lyase and malate synthetase
34.	Which of the pathway is involved with sprouting of seeds	Glycolysis	Glyoxylate cycle	Glycogenesis	glycogenolysis	Glyoxylate cycle
35.	In hydration, fumarate is converted by fumarase to	L-Malate	D-Malate	A-Malate	C-Malate	L-Malate
36.	Which of the following is a multi	Isocitrate	Alpha keto glutarate	Succinate	Malate	Alpha keto

	complex enzyme	dehydrogenase	dehydrogenase	dehydrogenase	dehydrogenase	glutarate
37.	In gluconeogenes, glucose is generated by	Non carbohydrate carbon substrates	Carbohydrate carbon substrates	Sucrose	Yeast	Non carbohydrate
38.	Gluconeogenesis is often associated with	Ketosis	Hexoses	Pentoses	Aldolase	Ketosis
39.	In vertebrates, gluconeogenesis mainly takes place in	Stomach	Liver	Heart	Intestine	Liver
40.	Number of enzyme catalyzed reactions in gluconeogenesis are	12	13	11	10	11
41.	The first two intermediates in the process of glycolysis are, respectively	Glucose 6-phosphate and glucose 1-phosphate	Glucose 1-phosphate and glucose 6-phosphate	Glucose 6-phosphate and fructose 6-phosphate	Glucose 1-phosphate and fructose 1-phosphate	Glucose 6-phosphate and fructose 6-phosphate
42.	The name of the process in which glucose 6-phosphate is converted to glycogen is	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis	Glycogenesis
43.	Which of the following processes requires UTP molecules?	Formation of glycogen from glucose 6-phosphate	Degradation of glycogen to glucose 6-phosphate	Formation of glucose 1-phosphate from glucose 6-phosphate	Degradation of glucose 1-phosphate from glucose 6-phosphate	Formation of glycogen from glucose 6-phosphate
44.	Glycogen is converted to glucose in which of the following processes?	Gluconeogenesis	Glycogenesis	Glycogenolysis	Glycolysis	Glycogenolysis
45.	Which of the following intermediates is not involved in glycolysis but is in gluconeogenesis?	Fructose 6-phosphate	Pyruvate	Oxaloacetate	Glicose-6 -phosphate	Oxaloacetate
46.	Which is not a substrate for gluconeogenes?	Lipids	proteins	Glucose	Lipids and proteins	Lipids and proteins
47.	In which of the following processes glucose 1-phosphate is released?	Glycogenesis	Glycogenolysis	Glycolysis	Citric acid cycle	Glycogenolysis

48.	The compound glucose 1-phosphate is encountered in which of the following processes?	Glycogenesis and glycogenolysis	Glycolysis and gluconeogenesis	Glycogenesis and gluconeogenesis	Glycolysis and glycogenolysis	Glycogenesis and glycogenolysis
49.	The main organ involved with the maintenance of blood glucose is	Liver	kidney	brain	Adipose tissue	Liver
50.	In the human body, under aerobic conditions and anaerobic conditions, pyruvate is converted to____ and ____ respectively,	Lactate and ethanol	Lactate and acetyl Coa	Ethanol and lactate	Acetyl CoA and lactate	Acetyl CoA and lactate
51.	The general chemical formula of carbohydrate is	$(CH_2O)n$	$(CH_2O)2n$	(CHO)n	CnH_2nO	$(CH_2O)n$
52.	Which of the following is a debranching enzyme	Glycogen synthetase	Glucose- 6-Phpsphatase	Amylo (1,6) glucosidase	Amylo 1,4-1,6	Amylo (1,6) glucosidase
53.	Which of the following is a branching enzyme	Glycogen synthetase	Glucose- 6-Phpsphatase	Amylo (1,6) glucosidase	Amylo 1,4-1,6	Amylo (1,6)
54.	Majority of the monosaccharides found in the human body are of …	L-type	D-type	DL-types	LD-types	D-type
55.	The sugar found in DNA is	Xylose	Ribose	Deoxyribose	Ribulose	Deoxyribose
56.	The enzyme present in liver that convert glucose -6 -phosphate to glucose	Glyceraldehyde-3 phosphate-dehydrogenase	Glucose-6-phosphatedehydrogenase	Glucose-6-phosphatase	Phospho hexose isomerase	Glucose-6-phosphatase
57.	Which of the following enzyme is not involved in HMP shunt?	Glyceraldehyde-3 phosphate-dehydrogenase	Glucose-6-phosphatedehydrogenase	Transketolase	Phosphogluconate dehydrogenase	Glyceraldehyde-3-phosphatedehydrogenase
58.	Which of the following is a substrate for aldolase activity in Glycolytic pathway?	Glyceraldehyde-3-phosphate	Glucose-6-phosphate	Fructose-6-phosphate	Fructose1, 6-bisphosphate	Fructose1, 6-bisphosphate
59.	An allosteric enzyme responsible for controlling the rate of T.C.A cycle is	Malate dehydrogenase	Isocitrate dehydrogenase	Fumarase	Aconitase	Isocitrate dehydrogenase

60.	Glyoxylate cycle involves with	Glyoxysome, lipid body	Glyoxysome, lipid body and mitochondria	Glyoxysome, and mitochondria	lipid body and mitochondria	Glyoxysome, lipid body and mitochondria

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DEPARTMENT OF BIOCHEMISTRY

SUBJECT : METABOLIC PATHWAYS
SEMESTER : V
SUBJECT CODE : 15BCU501 CLASS : III B.Sc.BC

UNIT III-- COURSE MATERIAL

Metabolism of lipids

Introduction- Blood lipids and plasma lipoproteins- biomedical importance, fate of dietary lipids. Oxidation of Fatty acids: β- oxidation, α-oxidation and ω oxidation. Oxidation of fatty acids with odd numbers of carbon atoms. Biosynthesis of saturated fatty acids: Extra mitochondrial and microsomal system for elongation of fatty acids. Biosynthesis of Phospholipids: Phosphatidyl choline, Phosphatidyl ethanolamine, Phosphatidyl inositol and Phosphatidyl serine. Degradation of phospholipids, Biosynthesis of glycolipids, Biosynthesis of Cholesterol. Ketone bodies – formation, importance.

TEXT BOOKS

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Robert K. Murray, Daryl K. Granner Peter A. Mayes Victor W. Rodwell, 2012, Harper's Biochemistry, 29th Ed, Mc Graw-Hill Medical, London.

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UNIT-III: METABOLISM OF LIPIDS

Introduction:

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. The biological functions of the lipids are as diverse as their chemistry. Fats and oils are the principal stored forms of energy in many organisms. Phospholipids and sterols are major structural elements of biological membranes. Other lipids, although present in relatively small quantities, play crucial roles as enzyme cofactors, electron carriers, light absorbing pigments, hydrophobic anchors for proteins, "chaperones" to help membrane proteins fold, emulsifying agents in the digestive tract, hormones, and intracellular messengers.

Lipids are indispensable for cell structure and function. Due to their hydrophobic and nonpolar nature, lipids differ from rest of the body compounds and are unique in their action

Triacylglycerols, the body fuel reserve

Lipids constitute about "15-20% of the body weight in humans. Blood lipids (or blood fats) are lipids in the blood, either free or bound to other molecules. Blood lipids are mainly fatty acids and cholestero Triacylglycerols (formerly triglycerides) are the most abundant lipids comprising 85-90% of body lipids. Most of the triacylglycerols (TC; also called neutral fat or depot fat) are stored in the adipose tissue and serve as energy reserve of the body. This is in contrast to carbohydrates and proteins which cannot be stored to a significant extent for energy purposes. Fat also acts as an insulating material for maintaining the body temperature of animals Triacylglycerols are the most predominant storage form of energy. There are two main reasons for fat being the fuel reserve of the body

1. Triacylglycerols (TC) are highly concentrated form of energy, yielding 9 Cal/g, in contrast to carbohydrates and proteins that produce only 4 Cal/g. This is because fatty acids found in TG are in the reduced form.
2. The triacylglycerols are non-polar and hydrophobic in nature, hence stored in pure form without any association with water (anhydrous form). On the other hand, glycogen and proteins are polar. One gram of glycogen combines with 2 g of water for storage.

For the two reasons stated above, one gram of fat stored in the body yields nearly six times as much energy as one gram of (hydrated) glycogen. Fats can support the body's energy needs for

long periods of food deprivation. In extreme cases, humans can fast and survive for 60-90 days, and the obese persons can survive even longer (6 months to one year) without food.

Other important body lipids

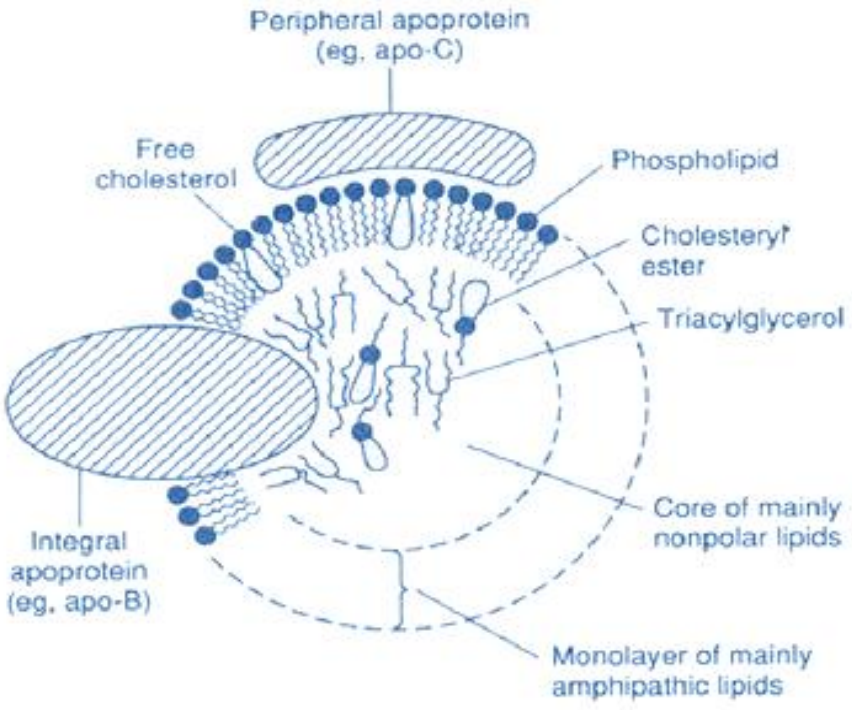
Phospholipids, glycolipids and cholesterol are major components of cell membranes. Cholesterol is also a precursor for bile acids and steroid hormones. Arachidonic acid an unsaturated fatty acid is the substrate for the synthesis of certain intercellular regulators prostagalndins, thromboxanes, prostacycilns etc.

Transport of Lipids

The insoluble lipids are solubilized in association with proteins to form lipoproteins in which form lipids are transported in the blood stream. Free lipids are undetectable in blood. Chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and albumin-free fatty acids are the different lipoprotein complexes that transport lipids in the blood stream.

Lipoproteins

Lipoproteins are molecular complexes that consist of lipids and proteins (conjugated proteins). They function as transport vehicles for lipids in blood plasma. Lipoproteins deliver the lipid components (cholesterol, triacylglycerol etc.) to various tissues for utilization.



Five major classes of lipoproteins are identified in human plasma, based on their separation by electrophoresis.

1. Chylomicrons: They are synthesized in the intestine and transport exogenous (dietary) triacylglycerol to various tissues. They consist of highest (99%) quantity of lipid and lowest (1%) concentration of protein. The chylomicrons are the least in density and the largest in size, among the lipoproteins.
2. Very low density lipoproteins (VLDL): They are produced in liver and intestine and are responsible for the transport of endogenously synthesized triacylglycerols.
3. Low density lipoproteins (LDL): They are formed from VLDL in the blood circulation. They transport cholesterol from liver to other tissues.
4. High density lipoproteins (HDL): They are mostly synthesized in liver. Three different fractions of HDL (1, 2 and 3) can be identified by ultracentrifugation HDL particles transport cholesterol from peripheral tissues to liver (reverse cholesterol transport).
5. Free fatty acids-albumin: Free fatty acids in the circulation are in a bound form to albumin. Each molecule of albumin can hold about 20-30 molecules of free fatty acids. This lipoprotein cannot be separated by electrophoresis.

Apolipoproteins (apoproteins)

The protein components of lipoproteins are known as apolipoproteins or, simply, apoproteins. They perform the following functions

1. Act ass tructuracl omponentso f lipoproteins.
2. Recognize the cell membrane surface receptors.
3. Activate enzymes involved in lipoprotein metabolism.

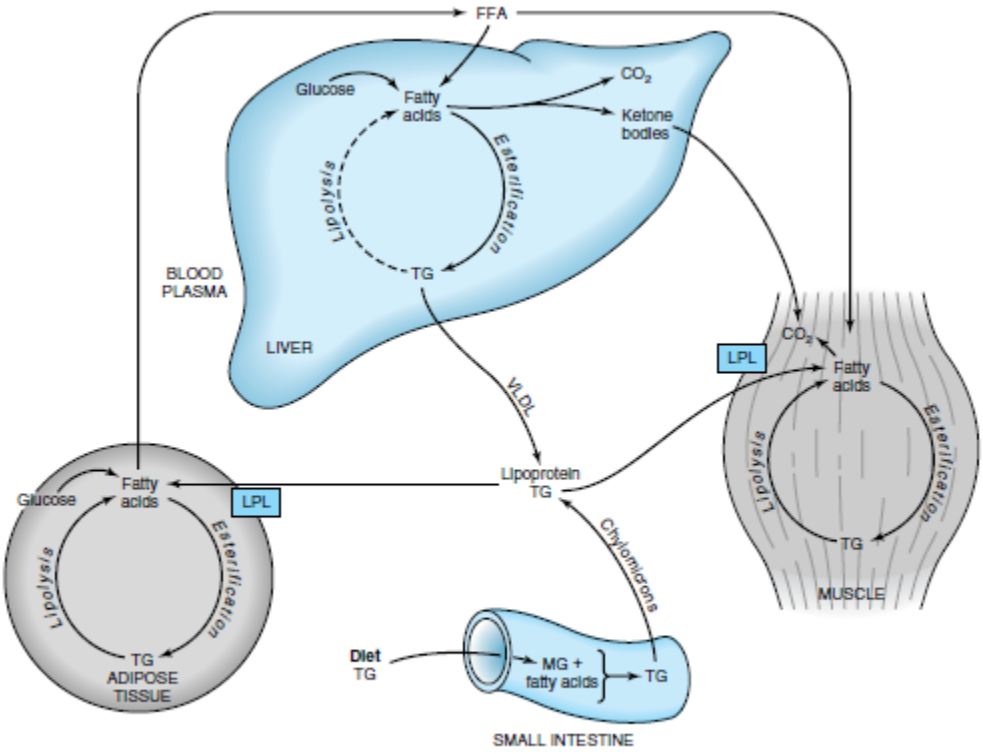
Biomedical Importance

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. They have the common property of being (1) relatively insoluble in water and (2) soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization

waves along myelinated nerves. Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria, and serving also as the means of transporting lipids in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, eg, obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

Fate of dietary lipids

Lipids in the diet are mainly triacylglycerol and are hydrolyzed to monoacylglycerols and fatty acids in the gut, then reesterified in the intestinal mucosa. Here they are packaged with protein and secreted into the lymphatic system and thence into the blood stream as **chylomicrons,** the largest of the plasma **lipoproteins.** Chylomicrons also contain other lipidsoluble nutrients, eg, vitamins. Unlike glucose and amino acids, chylomicron triacylglycerol is not taken up directly by the liver. It is first metabolized by tissues that have **lipoprotein lipase,** which hydrolyzes the triacylglycerol, releasing fatty acids that are incorporated into tissue lipids or oxidized as fuel. The other major source of long-chain fatty acid is synthesis **(lipogenesis)** from carbohydrate, mainly in adipose tissue and the liver.

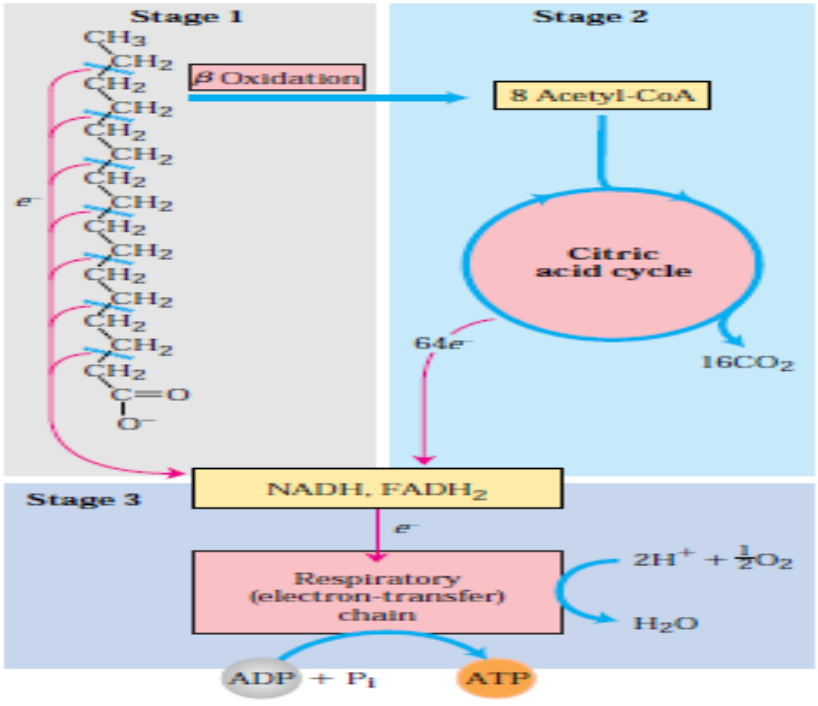


Transport and fate of major lipid substrates and metabolites. (FFA, free fatty acids; LPL, lipoprotein lipase; MG, monoacylglycerol; TG, triacylglycerol; VLDL, very low density lipoprotein.)

Adipose tissue triacylglycerol is the main fuel reserve of the body. On hydrolysis **(lipolysis)** free fatty acids are released into the circulation. These are taken up by most tissues (but not brain or erythrocytes) and esterified to acylglycerols or oxidized as a fuel. In the liver, triacylglycerol arising from lipogenesis, free fatty acids, and chylomicron remnants is secreted into the circulation as **very low density lipoprotein** (VLDL). This triacylglycerol undergoes a fate similar to that of chylomicrons. Partial oxidation of fatty acids in the liver leads to **ketone body** production (keto- genesis). Ketone bodies are transported to extrahepatic tissues, where they act as a fuel source in starvation.

Oxidation of fatty acid

Mitochondrial oxidation of fatty acids takes place in three stages. In the first stage β-oxidation-fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16-carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two carbons of palmitate (originally C-15 and C-16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H^+) from the fatty acyl moiety by dehydrogenases.



Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA.

Stage 2: The acetyl residues are oxidized to CO2 via the citric acid cycle.

Stage 3: Electrons derived from the oxidations of Stages 1 and 2 are passed to O2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

In the second stage of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation. The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and $FADH_2$, which in the third stage donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP.

The *β- o*xidation of Saturated Fatty Acids

a)Fatty acid activation

Fatty acids are oxidized inside the mitochondrial matrix but the fatty acids to be oxidized come from the cytosol. Fatty acids are activated in the cytosol by esterification with Coenzyme A (CoA) to form acyl-CoA (RCO-CoA, where R is the fatty acid acyl group).

Activated medium-chain fatty acids (C8 and C10) freely diffuse into mitochondria to be oxidized but long chain fatty acids do not diffuse into mitochondria so they must be transported in.

b) Trnasport of fattyacid into Mitochondria

The carnitine shuttle is responsible for transferring long-chain fatty acids across the barrier of the inner mitochondrial membrane to gain access to the enzymes of beta-oxidation. The carnitine shuttle consists of three enzymes (carnitine palmitoyltransferase 1 (CPT1A and CPT1B) , carnitine acylcarnitine translocase (SLC25A20), carnitine palmitoyl-transferase 2 (CPT2)) and a small, soluble molecule, carnitine, to transport fatty acids as their long-chain fatty acylcarnitine esters.

The transport of long chain fatty acids into mitochondria for oxidation is accomplished by the carnitine palmitoyltransferase system (CPTI and CPTII). CPTI exchanges carnitine for the CoA attached to long chain fatty acids to form a fatty acid-carnitine conjugate (RCO-carnitine).

The fatty acid-carnitine is transported into the matrix by a transporter protein in the inner mitochondrial membrane.

Once the fatty acid-carnitine is inside the matrix, CPTII exchanges CoA for carnitine to produce fatty acid-CoA once again, ready to enter fatty acid oxidation in the matrix to produce energy. The free carnitine is transported back out to renew the cytoplasmic pool of carnitine and allow the transfer process to continue.

c) *β* oxidation of fatty acid

Four enzyme-catalyzed reactions make up the first stage of fatty acid oxidation. First, dehydrogenation of fatty acyl–CoA produces a double bond between the *α* and *β* carbon atoms (C-2 and C-3), yielding a ***trans*-Δ^2-enoyl-CoA** (the symbol Δ^2designates the position of the double bond) the new double bond has the trans configuration, whereas the double bonds in naturally occurring unsaturated fatty acids are normally in the cis configuration.

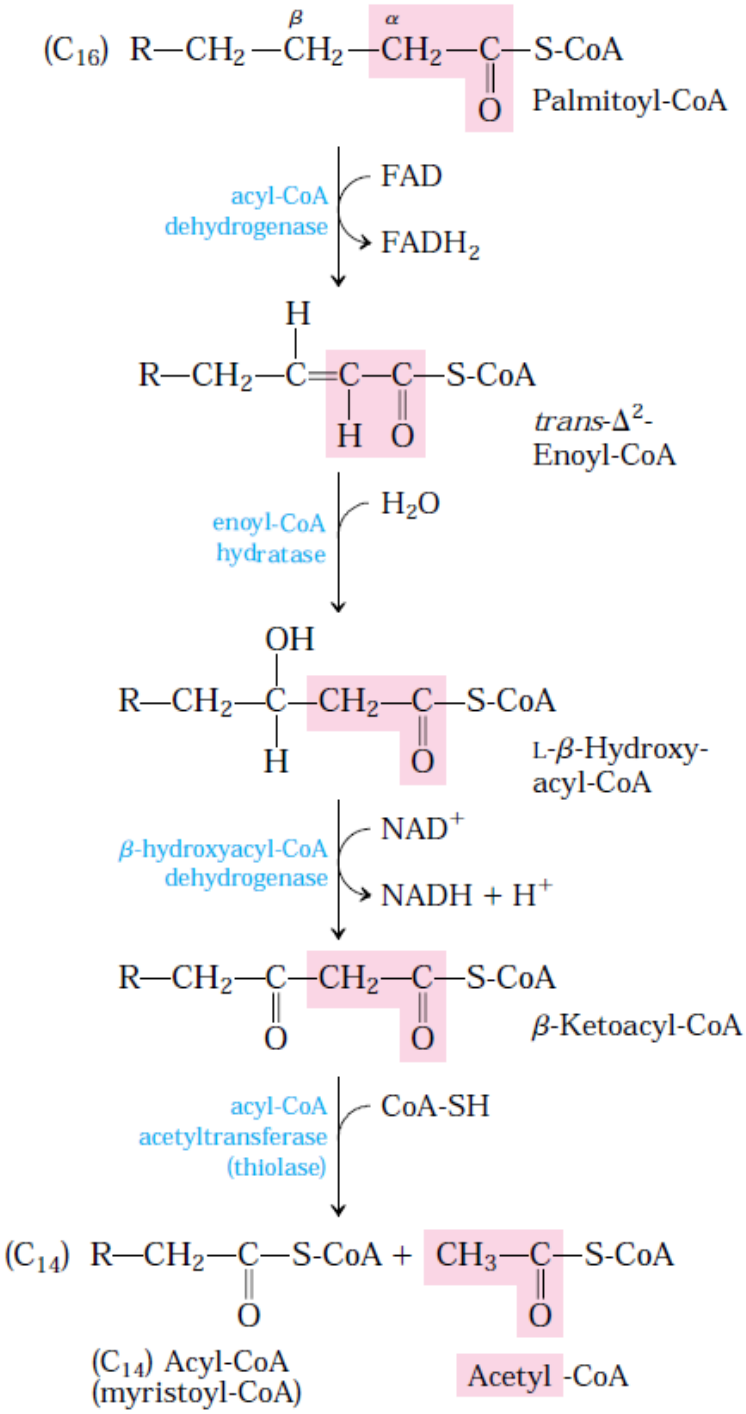
This first step is catalyzed by three isozymes of **acyl-CoA dehydrogenase,** each specific for a range of fatty-acyl chain lengths: very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons; medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons; and short-chain (SCAD), acting on fatty acids of 4 to 8 carbons. All three isozymes are flavoproteins with FAD as a prosthetic group. The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the **electron-transferring flavoprotein (ETF)**. The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle; in both reactions the enzyme is bound to the inner membrane, a double bond is introduced into a carboxylic acid between the *α* and *β* carbons, FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O_2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

In the second step of the *β* -oxidation cycle, water is added to the double bond of the ***trans*-Δ^2-enoyl-CoA** to form the L stereoisomer of ***β* -hydroxyacyl-CoA (3-hydroxyacyl-CoA).** This reaction, catalyzed by **enoyl-CoA hydratase,** is formally analogous to the fumarase reaction in the citric acid cycle, in which H_2O adds across an *α* -*β* double bond.

In the third step, L- *β* -hydroxyacyl-CoA is dehydrogenated to form *β* **-ketoacyl-CoA,** by the action of *β* **-hydroxyacyl-CoA dehydrogenase;** NAD^+ is the electron acceptor. This enzyme

is absolutely specific for the L stereoisomer of hydroxyacyl-CoA. The NADH formed in the reaction donates its electrons to **NADH dehydrogenase,** an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O_2. The reaction catalyzed by β - hydroxyacyl-CoA dehydrogenasen is closely analogous to the malate dehydrogenase reaction of the citric acid cycle.

The fourth and last step of the β-oxidation cycle is catalyzed by **acyl-CoA acetyltransferase,** more commonly called **thiolase,** which promotes reaction of β - ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms. This reaction is called thiolysis, by analogy with the process of hydrolysis, because the β -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.



The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain. For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the **trifunctional protein (TFP).** TFP is a

heterooctamer of $\alpha_4\beta_4$ subunits. Each α subunit contains two activities, the enoyl-CoA hydratase and the β -hydroxyacyl-CoA dehydrogenase; the β -subunits contain the thiolase activity. This tight association of three enzymes may allow efficient substrate channeling from one active site to the next, without diffusion of the intermediates away from the enzyme surface. When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

The β -oxidation sequence is an elegant mechanism for destabilizing and breaking these bonds. The first three reactions of β -oxidation create a much less stable C-C bond, in which the α_carbon (C-2) is bonded to *two* carbonyl carbons (the β -ketoacyl-CoA intermediate). The ketone function on the β- carbon (C-3) makes it a good target for nucleophilic attack by the -SH of coenzyme A, catalyzed by thiolase. The acidity of the α -hydrogen and the resonance stabilization of the carbanion generated by the departure of this hydrogen make the terminal -CH2-CO-S-CoA a good leaving group, facilitating breakage of the α– β bond.

Energetic of β –oxidation

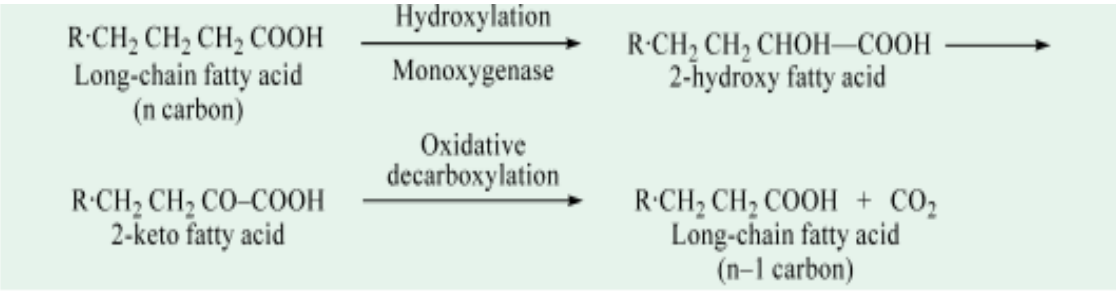
Palmitic acid (16 carbons) undergoes 7 times β-oxidation and produces 8 molecules of acetyl-CoA. Each time, β-oxidation produces 5 ATP.

Total number of ATP formed through β-oxidation	7 X5 =35
Total number of ATP formed on oxidation of acetyl-CoA through TCA cycle	8 X12 =96
Total	131
2ATP utilised for initial activation of Fatty acid	-2
Net Total yield	129 ATP

α-Oxidation of fatty acids

Although β oxidation is major pathway for the oxidation of fatty acids, two other types of oxidation also occur, α and ω oxidation. α oxidation is the removal of one carbon atom (i.e., α carbon) at a time from the carboxyl end of the molecule. α oxidation was first observed in seeds and leaf tissues of plants. α oxidation of long-chain fatty acids to 2-hydroxy acids and then to fatty acids with one carbon atom less than the original substrate have been demonstrated in the microsomes of brain and other tissues also. Long-chain α hydroxy fatty acids are constituents of brain lipids, e.g., the C_{24} cerebronic acid (= 2 hydroxylignoceric acid), CH_3 $(CH_2)_{21}$. CH(OH).

COOH. These hydroxy fatty acids can be converted to the 2-keto acids, followed by oxidative decarboxylation, resulting in the formation of long-chain fatty acids with an odd number of carbon atoms:



The initial hydroxylation reaction is catalyzed by a mitochondrial enzyme, monoxygenase that requires O_2, Mg^{2+}, NADPH and a heat-stable cofactor. Conversion of the α hydroxy fatty acid to CO_2 and the next lower unsubstituted acid appears to occur in the endoplasmic reticulum and to require O_2, Fe^{2+} and ascorbate.

The salient features of α oxidation are as follows:

1. Only free long-chain fatty acids serve as substrates.
2. Molecular oxygen is indirectly involved.
3. It does not require CoA intermediates.
4. It does not lead to generation of high-energy phosphates.

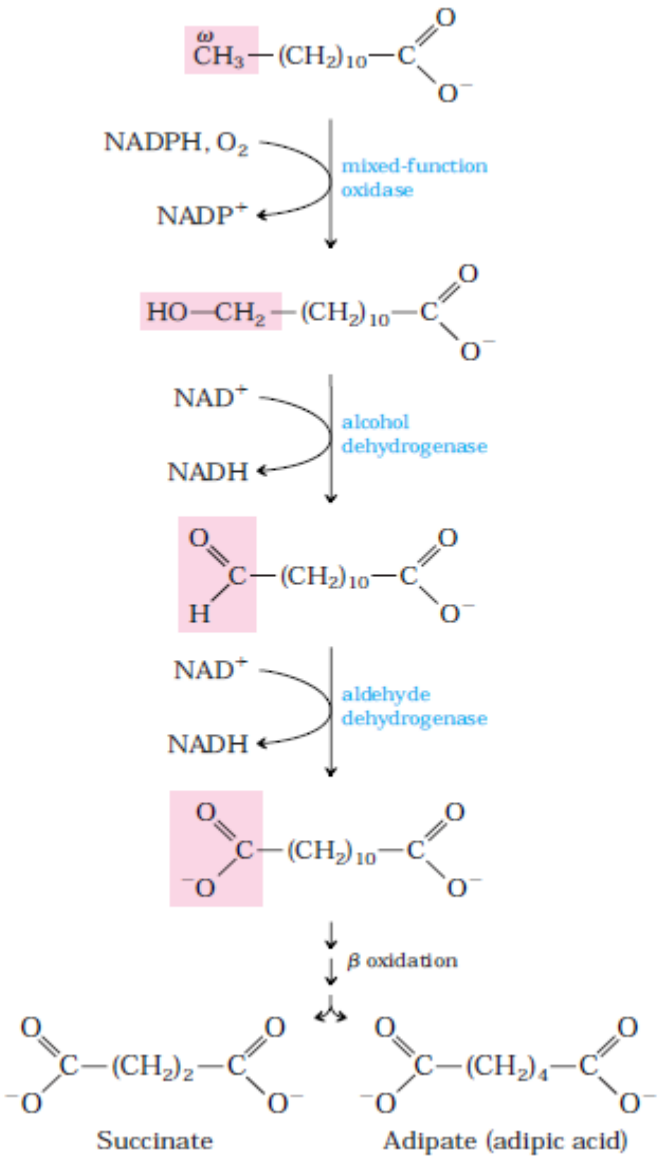
This mechanism explains the occurrence of α hydroxy fatty acids and of odd-numbered fatty acids in the biomolecules. The latter may, in nature, also be synthesized de novo from propionate. The α oxidation system plays a key role in the capacity of mammalian tissues to oxidize phytanic acid (= 3,7,11,15-tetramethylhexadecanate). Phytanic acid is an oxidation product of phytol and is present in animal fat, cow's milk and foods derived from milk. The phytol presumably originates from plant sources, as it is a substituent of chlorophyll and the side chain of vitamin K_2. Normally, phytanic acid is rarely found in serum lipids because of the ability of normal tissue to degrade (or oxidize) the acid very rapidly. But large amounts of phytanic acid accumulate (as much as 20% of the serum fatty acids and 50% of the hepatic fatty acids) in the tissues and serum of individuals with Refsum's disease, a rare inheritable autosomal recessive disorder affecting the nervous system because of an inability to oxidize this acid. Diets low in animal fat and milk products appear to relieve some of the symptoms of Refsum's disease. The presence of 3-methyl group in phytanic acid blocks β oxidation. In the mitochondria

of normal individuals, α hydroxylation of phytanic acid by phytanate α hydroxylase is followed by oxidation by phytanate α oxidase to yield CO_2 and pristanic acid (= 2,6,10, 14-tetramethylpentadecanoic acid), which readily undergoes β oxidation after conversion to its CoA derivative. In Refsum's disease, there is a lack of the enzyme, phytanate α hydroxylase.

ω- Oxidation of fatty acids

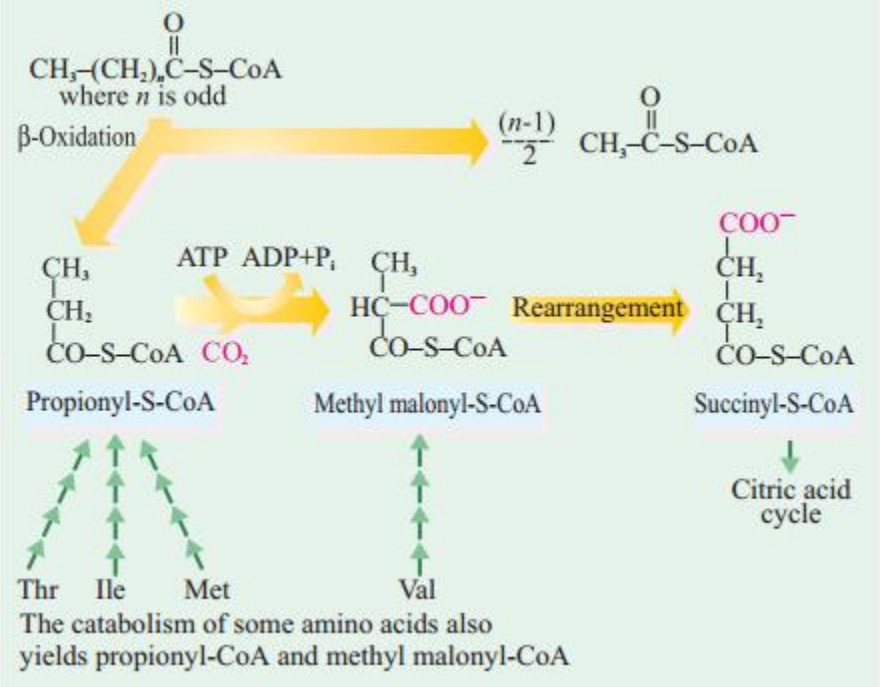
Although mitochondrial β oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of the ω (omega) carbon the carbon most distant from the carboxyl group. The enzymes unique to ω oxidation are located (in vertebrates) in the endoplasmic reticulum of liver and kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms. In mammals ω oxidation is normally a minor pathway for fatty acid degradation, but when β oxidation is defective (because of mutation or a carnitine deficiency, for example) it becomes more important.

The first step introduces a hydroxyl group onto the ω carbon. The oxygen for this group comes from molecular oxygen (O_2) in a complex reaction that involves cytochrome P450 and the electron donor NADPH. Reactions of this type are catalyzed by **mixedfunction oxidases,** described in Box 21–1. Two more enzymes now act on the ω carbon: **alcohol dehydrogenase** oxidizes the hydroxyl group to an aldehyde, and **aldehyde dehydrogenase** oxidizes the aldehyde group to a carboxylic acid, producing a fatty acid with a carboxyl group at each end. At this point, either end can be attached to coenzyme A, and the molecule can en ter the mitochondrion and undergo β oxidation by the normal route. In each pass through the β -oxidation pathway, the "double-ended" fatty acid yields dicarboxylic acids such as succinic acid, which can enter the citric acid cycle, and adipic acid



Oxidation of Odd-chain Fatty Acids

Most naturally-occurring lipids contain fatty acids with an even number of carbon atoms, yet fatty acids with an odd number of carbon atoms are found in significant amounts in the lipids of many plants and some marine animals. Small quantities of C-3 propionate are added as a mould inhibitor to some breads and cereals, and thus propionate enters the human diet. Besides, cattle and other ruminants form large amounts of propionate during fermentation of carbohydrates in the rumen. The propionate so formed is absorbed into the blood and oxidized by the liver and other tissues.

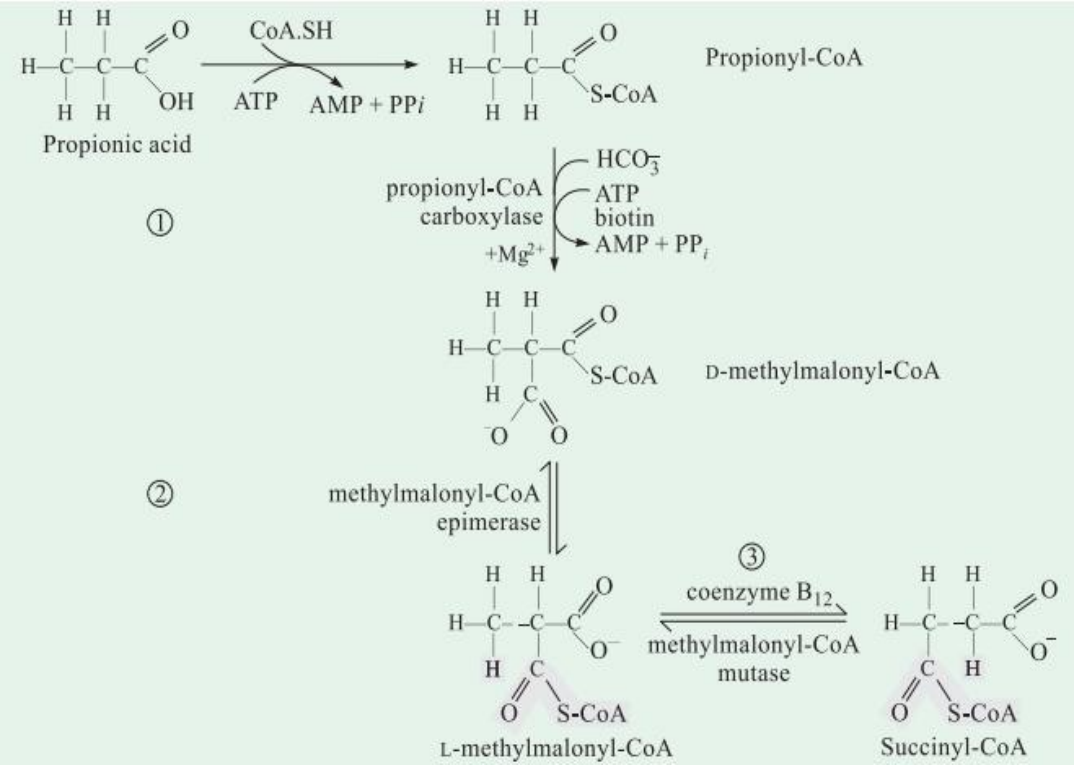


The odd-carbon long-chain fatty acids are oxidized by the same pathway as the even-carbon fattty acids, starting at the carboxyl end of the chain. However, the substrate for the last pass through the β oxidation cycle is a fatty acyl-CoA, in which the fatty acid has 5 carbon atoms. When this is oxidized and finally cleaved, the products are acetyl-CoA and propionyl CoA, rather than 2 moles of acetyl-CoA produced in the normal β oxidation cycle. The acetyl-CoA is, of course, oxidized via the citric acid cycle but the oxidation of propionylCoA presents an interesting problem, since at first glance the propionic acid (or propionylCoA) appears to be a substrate unsuitable for β oxidation. However, the substrate is held by two strikingly dissimilar pathways: methylmalonate pathway and β-hydroxy-propionate pathway

(a) Methylmalonate Pathway

This pathway is found only in animals and occurs in the mitochondria of liver, cardiac and skeletal muscles, kidney and other tissues. Propionate (or propionyl-CoA) is also produced by the oxidation of isoleucine, valine, methionine and threonine. Propionate is catalyzed by acetylCoA synthetase to produce propionyl-CoA. The propionyl-CoA is carboxylated to form the D stereoisomer of methylmalonyl-CoA by an enzyme propionyl-CoA carboxylase, which contains the cofactor biotin. In this reaction, as in pyruvate carboxylase reaction, the CO_2 (or its hydrated ion, HCO_3^-) is activated by attachment to biotin before its transfer to the propionate moiety. The formation of the carboxybiotin intermediate requires energy, which is provided by

the cleavage of ATP to AMP and PPi. The dmethylmalonyl-CoA, thus formed, is enzymatically epimerized to L-methylmalonyl-CoA, by the action of methylmalonyl-CoA epimerase (The epimerase labilizes the α-hydrogen atom, followed by uptake of a proton from the medium, thus catalyzing interconversion of D- and L-methylmalonylCoA). The L-methylmalonyl-CoA undergoes an intramolecular rearrangement to form succinylCoA by the enzyme methylmalonyl-CoA mutase, which requires as its coenzyme deoxyadenosyl-cobalamin or coenzyme B12. When [2–^{14}C] methyl-malonyl-CoA was converted by the mutase enzyme, the label (marked by an asterisk, below) was found in the 3 position of succinyl-CoA, thus indicating an intramolecular transfer of the entire thioester group, –CO–S– CoA, rather than migration of the carboxyl carbon.



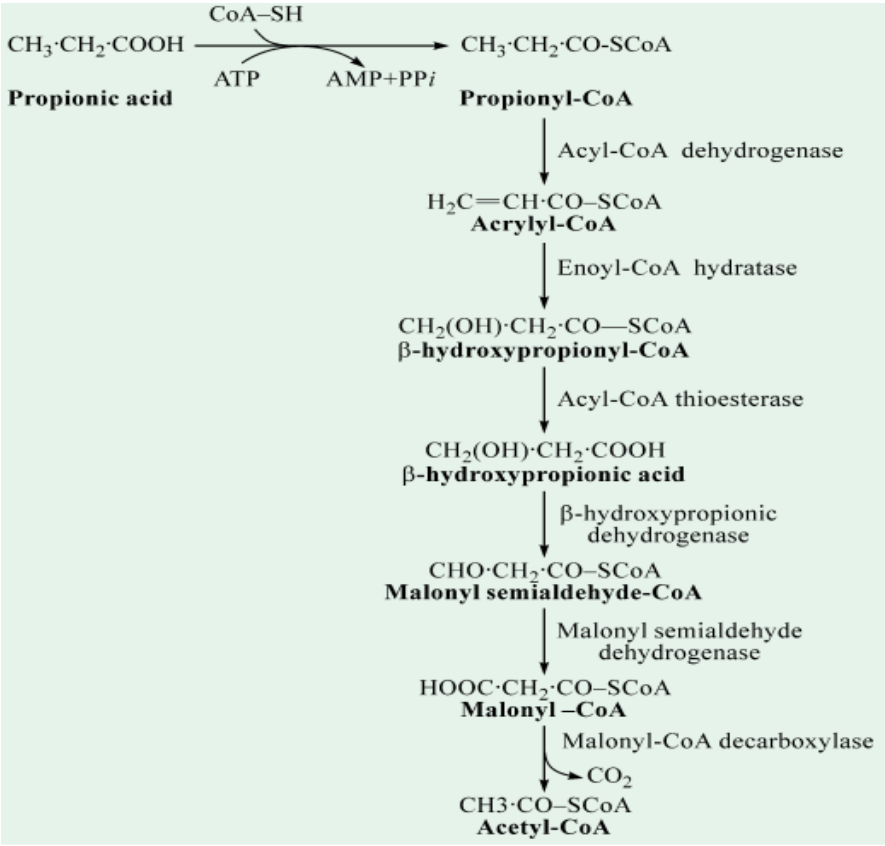
The role of the coenzyme B12 is to remove a hydrogen from one carbon atom by transferring it directly to an adjacent carbon atom, simultaneously effecting the exchange of a second (R) substituent. The H and R are not released into solution.

At equilibrium, formation of succinyl-CoA favoured by a ratio of 20: 1 over methylmalonyl-CoA. The succinyl-CoA can then be oxidized via succinate and the citric acid cycle to CO_2 and H_2O. In patients with vitamin B12 deficiency, both propionate and methylmalonate are excreted in the urine in abnormally large amounts. The odd-chain fatty acids are only a small fraction of the total, and only the terminal 3 carbons appear as propionyl-CoA.

The metabolism of propionyl-CoA is, therefore, not of quantitative significance in fatty acid oxidation.

(b) β-hydroxypropionate Pathway

This pathway is ubiquitous in plants and is a modified form of β oxidation scheme. It nicely resolves the problem of how plants can cope with propionic acid by a system not involving vitamin B12 as cobamide coenzyme. Since plants have no B12 functional enzymes, the methylmalonate pathway does not operate in them. This pathway, thus, bypasses the B12 barrier in an effective way.



BIOSYNTHESIS OF SATURATED FATTY ACIDS

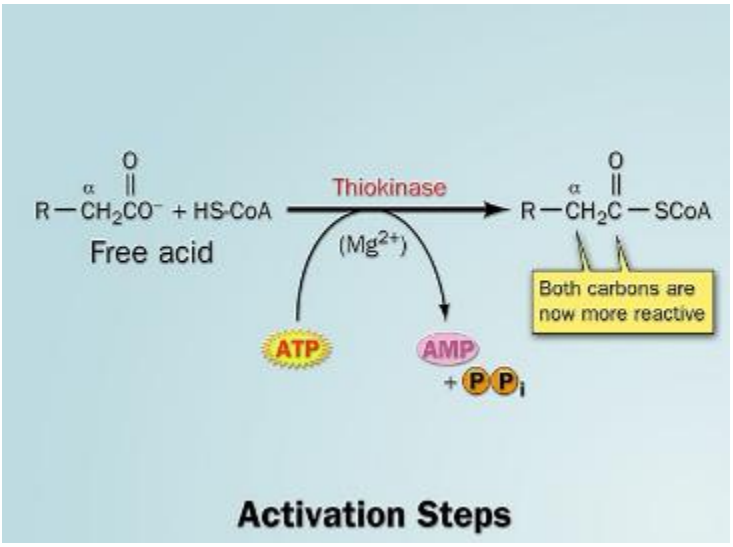
- Glucose entering the TCA cycle is used for the **biosynthesis of saturated fatty acids** by converting TCA Cycle citrate to acetyl coenzyme~A (acetyl~CoA), and then malonyl~CoA, which is used to produce palmitate.
- The glycerol backbone of TGs comes from glycolytic glycerol-3-phosphate.
- Triglycerides are the primary lipid synthesized, and serve as a starting point for other lipids such as steroids and phospholipids.

- *Biosynthesis of Saturated Fatty acids* primarily occurs in hepatocyte cytoplasm.
- Acetyl CoA and NADPH are both necessary for Biosynthesis of Saturated Fatty acids.

Steps involved in Biosynthesis of Saturated Fatty acids

Acetyl Coenzyme A:

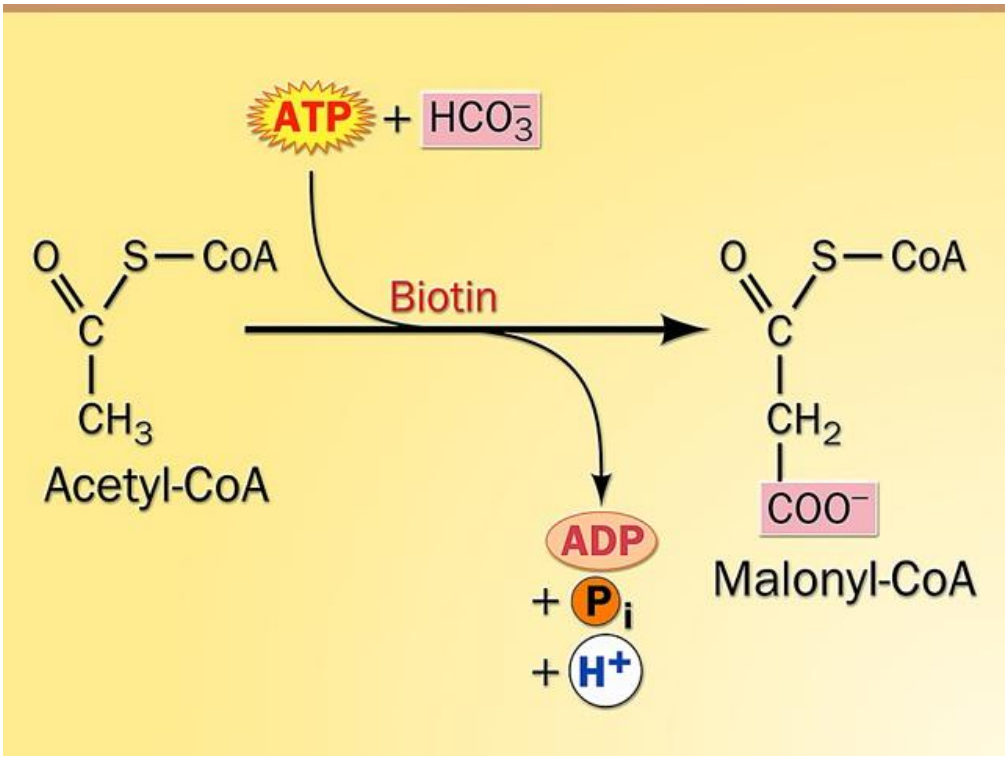
- Acetyl CoA is produced in the matrix of the mitochondria, but fatty acid biosynthesis occurs in the cytosol.
- Citrate synthase frees CoA from acetyl CoA and condenses acetate and oxaloacetate to citrate.
- Matrix membrane transporters for citrate move citrate to the cytosol, where it is acted upon by citrate lyase in the presence of CoA to re-form acetyl CoA and oxaloacetate.
- The oxaloacetate produced is converted to malate, and then to pyruvate, which is transported back to the mitochondrial matrix.
- The conversion of malate to pyruvate releases NADPH into the cytosol, which is necessary for fatty acid biosynthesis. (The hexose monophosphate shunt, pentose phosphate pathway, is the other major source for cytosolic NADPH.)



Synthesis of Malonyl Coenzyme A:

- Acetyl CoA, with the addition of CO_2, and with the hydrolysis of an ATP, is converted to malonyl CoA by acetyl CoA carboxylase (a biotin-dependent enzyme like all carboxylases).
- Acetyl CoA carboxylase (ACC) is, being the first enzyme in the fatty acid biosynthetic pathway, is a regulated enzyme.

- In the short term, **allosteric activation by citrate**, and allosteric inactivation by malonyl and palmitoyl CoAs, and covalent modification (phosphorylation and dephosphorylation) are the principal regulatory mechanisms.
- ACC is normally present as a tetrameric protomer (inactive form). The active form is the large polymer, which is favored by citrate binding and inhibited by malonyl and palmitoyl CoAs (products of the FA biosynthetic pathway).
- Phosphorylation is regulated by another mechanism, with glucagon and epinephrine activating PKA to phosphorylate (inactivate) ACC, and insulin activating phosphatase to re-activate the enzyme.
- The burden of long-term regulation is carried almost exclusively by up regulating the transcription of the enzyme itself.



Acetyl coA to Malonyl coA

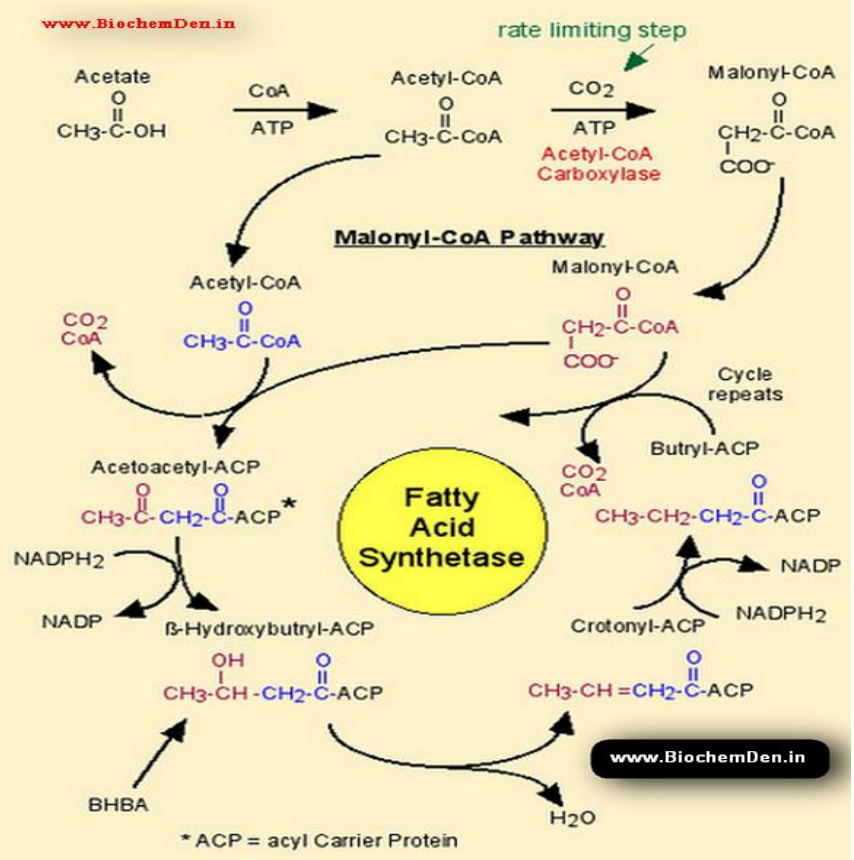
Fatty Acid Synthase Complex (FASC) Dimer:

- Seven enzymes and a "carrier" protein: acetyl CoA-ACP transacylase, malonyl CoA-ACP transacylase, β-ketoacyl-ACP synthase (condensing enzyme), β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydratase, enoyl-ACP reductase, palmitoyl thioesterase, and acyl carrier protein (ACP) (containsephosphopentetheine)

- The sulfhydryl group of one ACP unit associates with the **enoyl-ACP reductase** (ER) subunit of another FASC complex, allowing dimerization of the protein.
- ACP assists in reactions by binding to substrate molecules, such as acetate (from acetyl CoA) and malonate (from malonyl CoA).
- Any time a fatty acid is used in a *biosynthetic reaction in the cell*, it must be in the form of a fatty acyl CoA.

Steps in Biosynthesis of Saturated Fatty acids:

1. **Condensation:**
    - Acetate (2C) and malonate (3C), as acetyl-ACP and malonyl-ACP
    - Releases the non-ACP-bound carboxyl group of malonate as CO_2
    - Produces β-acetoacetyl-ACP (4C)
2. **Reduction:**
    - Produces β-hydroxybutyryl-ACP (4C), with the oxidation of $NADPH_2$ to $NADP^+$
3. **Dehydration:**
    - Produces crotonyl-ACP (4C) with the release of water
4. **Reduction:**
    - Produces Butyryl-ACP with the oxidation of $NADPH_2$ to $NADP^+$
5. **Repeat:**
    - Butyryl-ACP then enters into reaction 1 in the place of malonyl-ACP, undergoing the addition of another two carbons from acetate.
    - The overall reaction uses 8 acetyl CoA, 14 NADPH, 14 H^+ and 1 malonyl CoA to produce a 16-carbon palmitic acid.



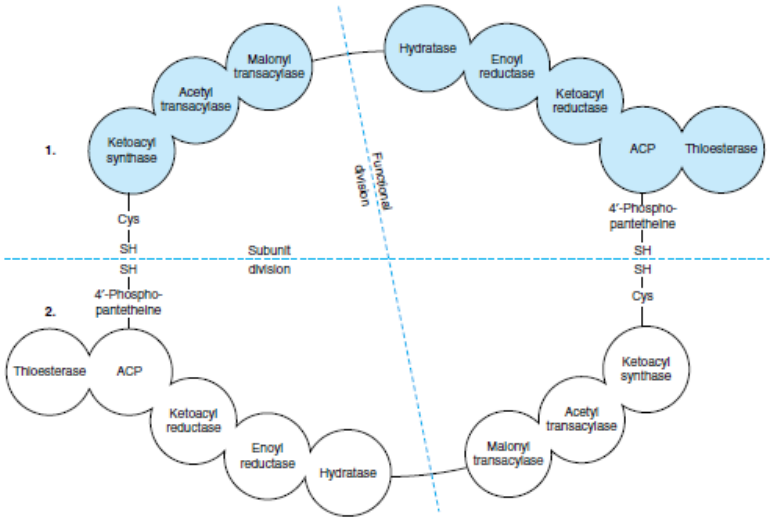
Fatty Acid Elongation:

- FASC produces palmitic acid (16C).
- In the endoplasmic reticulum, two-carbon units can be added to palmitate from malonyl CoA.
- In the mitochondria, two-carbon units can be added to 8C fatty acids from acetyl CoA, but only to the extent of 14C fatty acids.

The Fatty Acid Synthase Complex Is a Polypeptide Containing Seven Enzyme Activities

In bacteria and plants, the individual enzymes of the **fatty acid synthase** system are separate, and the acyl radicals are found in combination with a protein called the **acyl carrier protein (ACP).** However, in yeast, mammals, and birds, the synthase system is a multienzyme polypeptide complex that incorporates ACP, which takes over the role of CoA. It contains the vitamin **pantothenic acid** in the form of 4′-phosphopantetheine. The use of one multienzyme functional unit has the advantages of achieving the effect of compartmentalization of the process within the cell without the erection of permeability barriers, and synthesis of all enzymes in the complex is coordinated since it is encoded by a single gene.

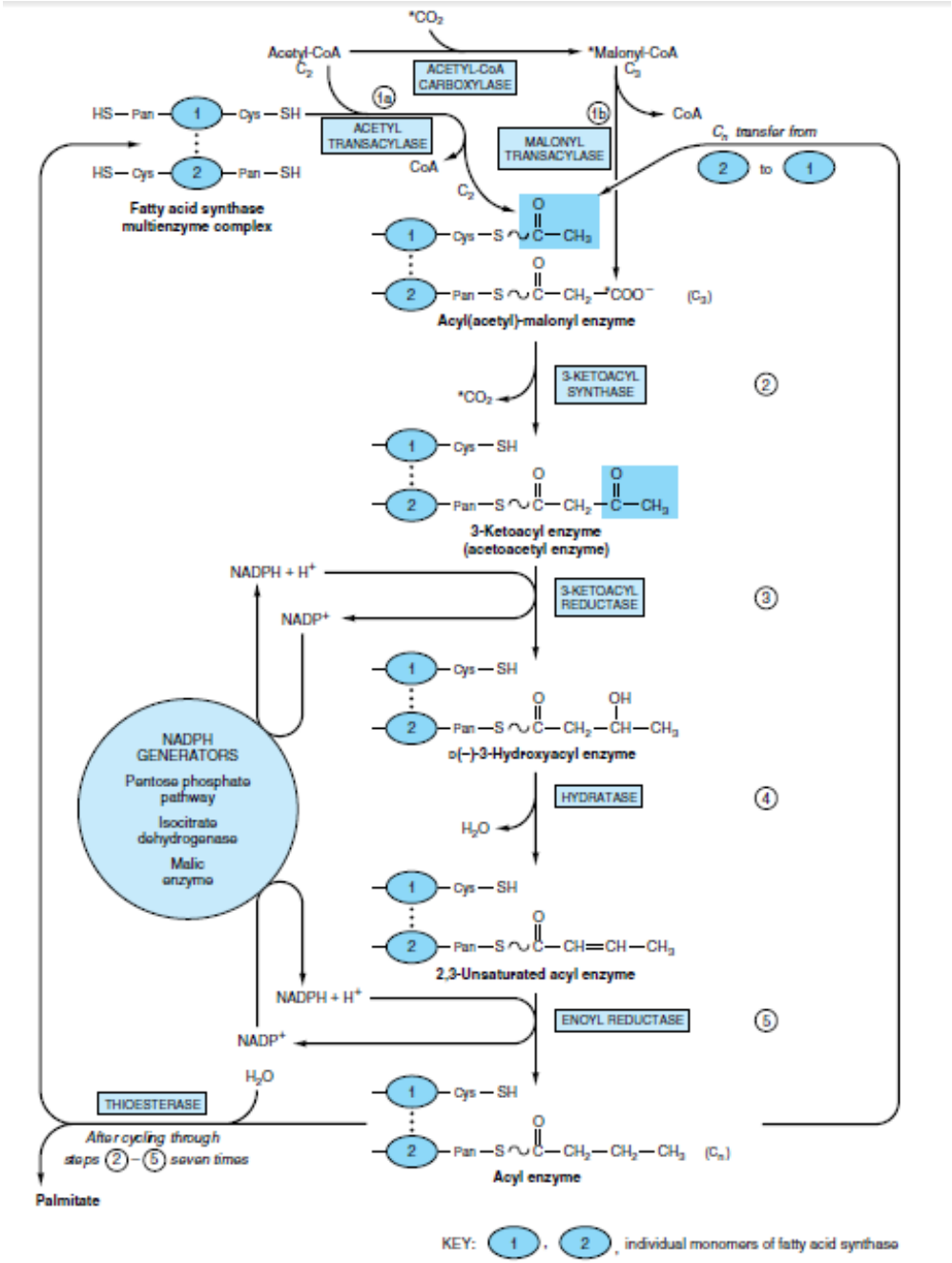
In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain.



Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers, 1 and 2, each consisting of seven enzyme activities and the acyl carrier protein (ACP). (Cys-SH, cysteine thiol.) The -SH of the 4′-phosphopantetheine of one monomer is in close proximity to the -SH of the cysteine residue of the ketoacyl synthase of the other monomer, suggesting a "head-to-tail" arrangement of the two monomers. Though each monomer contains all the partial activities of the reaction sequence, the actual functional unit consists of one-half of one monomer interacting with the complementary half of the other. Thus, two acyl chains are produced simultaneously. The sequence of the enzymes in each monomer is based on Wakil.

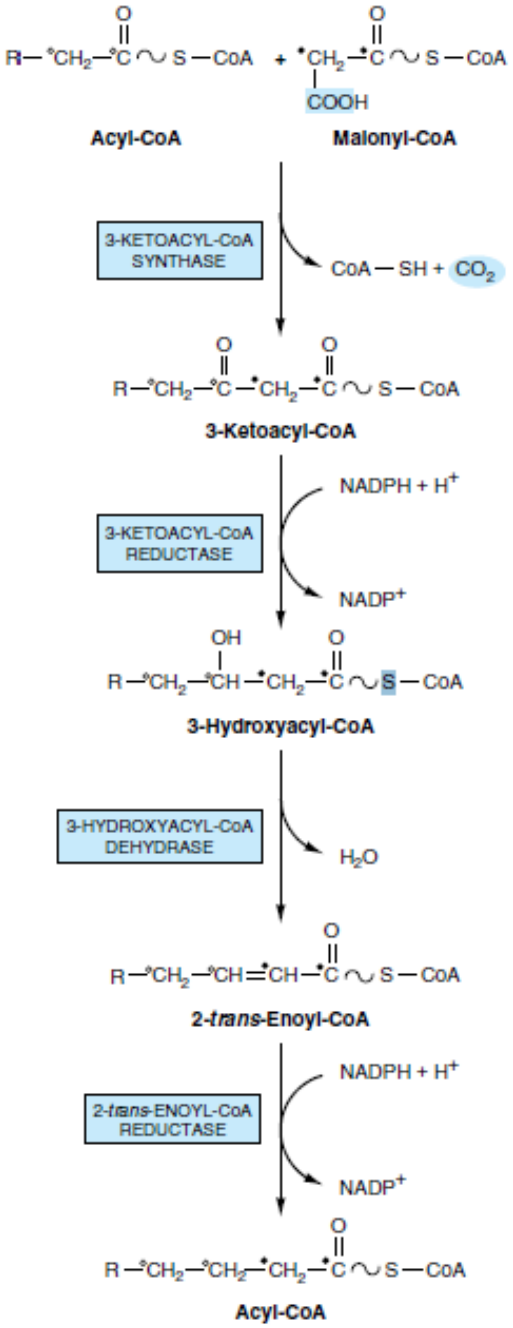
Initially, a priming molecule of acetyl-CoA combines with a cysteine-SH group catalyzed by **acetyl transacylase** Malonyl-CoA combines with the adjacent -SH on the 4′-phosphopantetheine of ACP of the other monomer, catalyzed by **malonyl transacylase**, to form **acetyl (acyl)-malonyl enzyme.** The acetyl group attacks the methylene group of the malonyl residue, catalyzed by **3-ketoacyl synthase,** and liberates CO2, forming 3-ketoacyl enzyme (acetoacetyl enzyme), freeing the cysteine -SH group. Decarboxylation allows the reaction to go to completion, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again to form the corresponding saturated acyl-Senzyme. A new malonyl-CoA molecule combines with the -SH of 4′-phosphopantetheine,

displacing the saturated acyl residue onto the free cysteine -SH group. The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmityl) has been assembled. It is liberated from the enzyme complex by the activity of a seventh enzyme in the complex, **thioesterase** (deacylase). The free palmitate must be activated to acyl-CoA before it can proceed via any other acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.



ELONGATION OF FATTY ACID CHAINS occurs in the endoplasmic reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as acetyl donor and NADPH as reductant, and is catalyzed by the microsomal **fatty acid elongase** system of enzymes (Figure). Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids for sphingolipids.



THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc, and to provide energy for use between meals in animals, including humans, that take their food at spaced intervals. Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The nutritional state of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake, on a fat diet, or when there is a deficiency of insulin, as in diabetes mellitus. These latter conditions are associated with increased concentrations of plasma free fatty acids, and an inverse relationship has been demonstrated between hepatic lipogenesis and the concentration of serum-free fatty acids. Lipogenesis is increased when sucrose is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway.

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

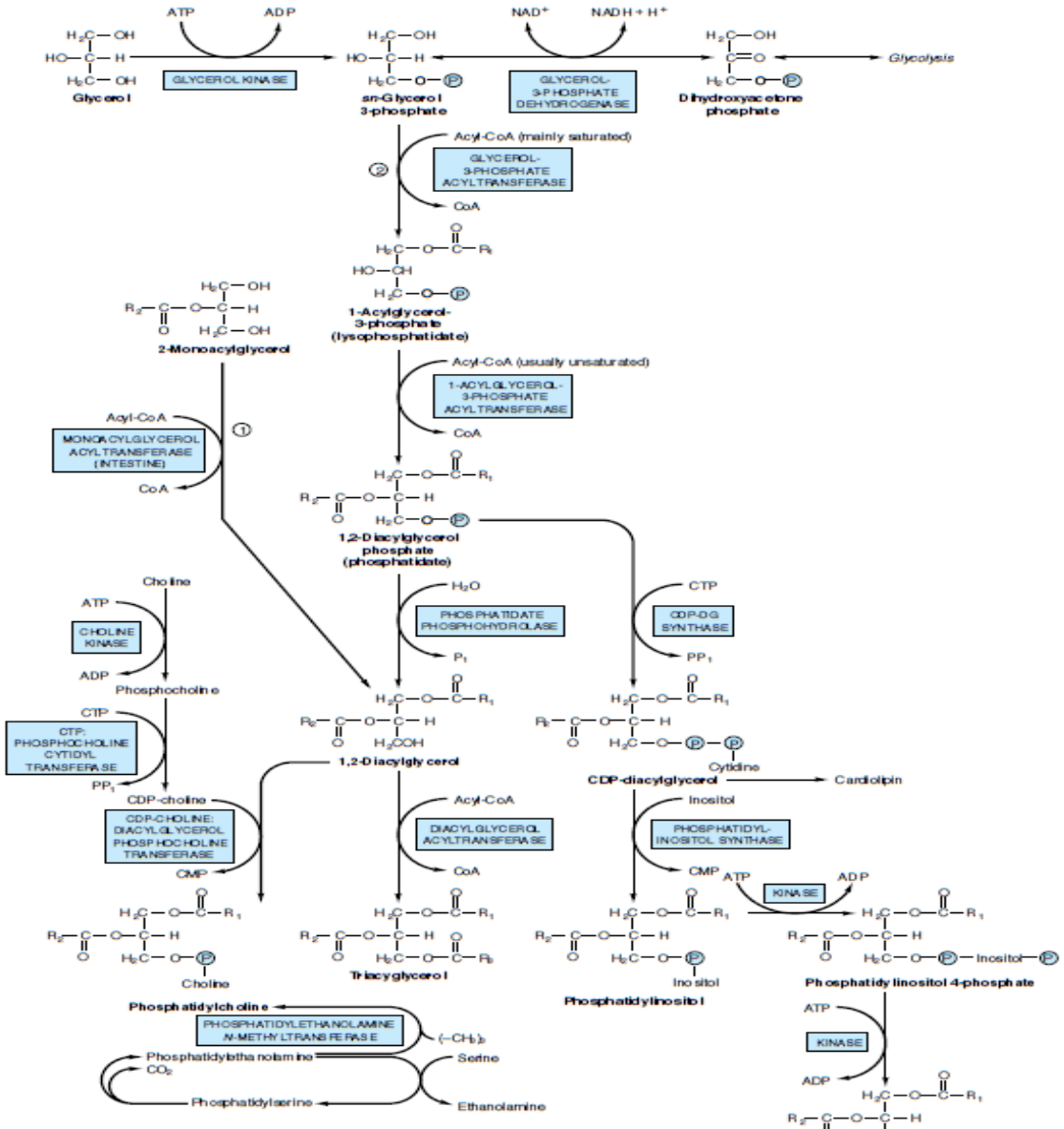
Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression governing rates of synthesis of enzymes.

BIOSYNTHESIS OF PHOSPHOLIPIDS

The diacylglycerol is activated by condensation of phosphatidic acid with cytidine triphosphate (CTP) to form CDP-diacylglycerol, with the elimination of pyrophosphate. Displacement of CMP through nucleophilic attack by the hydroxyl group of serine or by the C-1 hydroxyl of glycerol 3-phosphate yields **phosphatidylserine** or phosphatidylglycerol 3-phosphate, respectively. The latter is processed further by cleavage of the phosphate monoester (with release of P_i) to yield **phosphatidylglycerol.** Phosphatidylserine and phosphatidylglycerol can serve as precursors of other membrane lipids in bacteria. Decarboxylation of the serine moiety in phosphatidylserine, catalyzed by phosphatidylserine decarboxylase, yields **phosphatidylethanolamine.** In *E. coli*, condensation of two molecules of phosphatidylglycerol, with elimination of one glycerol, yields **cardiolipin,** in which two diacylglycerols are joined through a common head group.

Eukaryotes Synthesize Anionic Phospholipids from CDP-Diacylglycerol

In eukaryotes, phosphatidylglycerol, cardiolipin, and the phosphatidylinositols are synthesized by the same strategy used for phospholipid synthesis in bacteria. Phosphatidylglycerol is made exactly as in bacteria. Cardiolipin synthesis in eukaryotes differs slightly: phosphatidylglycerol condenses with CDP-diacylglycerol not another molecule of phosphatidylglycerol as in *E. coli*. Phosphatidylinositol is synthesized by condensation of CDP-diacylglycerol with inositol.

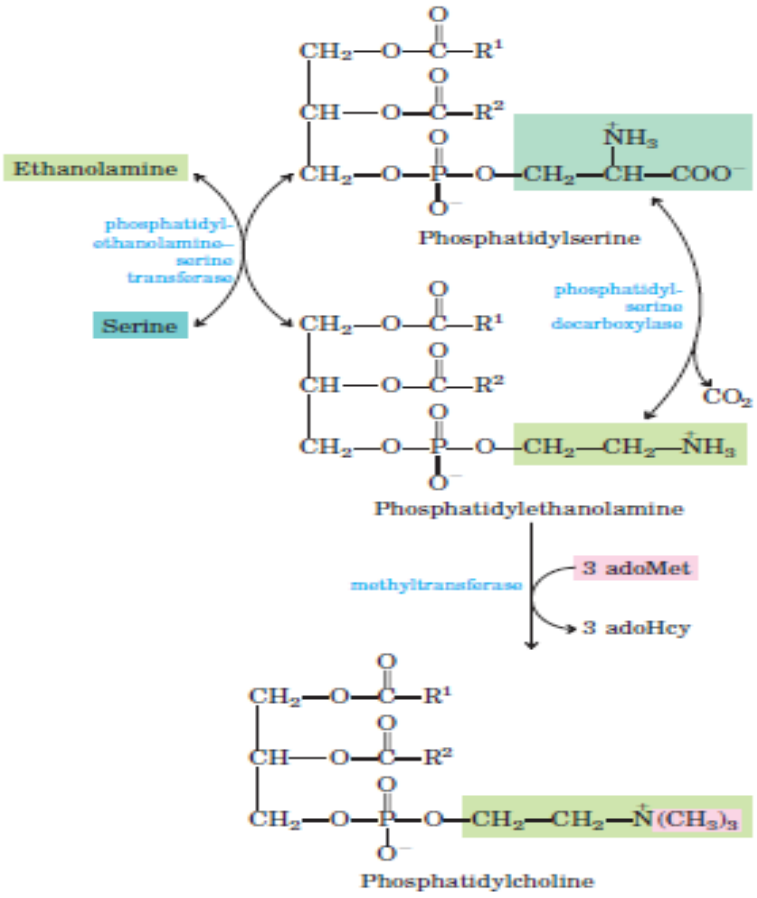


Biosynthesis of triacylglycerol and phospholipids. (①, Monoacylglycerol pathway; ②, glycerol phosphate pathway.) Phosphatidylethanolamine may be formed from ethanolamine by a pathway similar to that shown for the formation of phosphatidylcholine from choline.

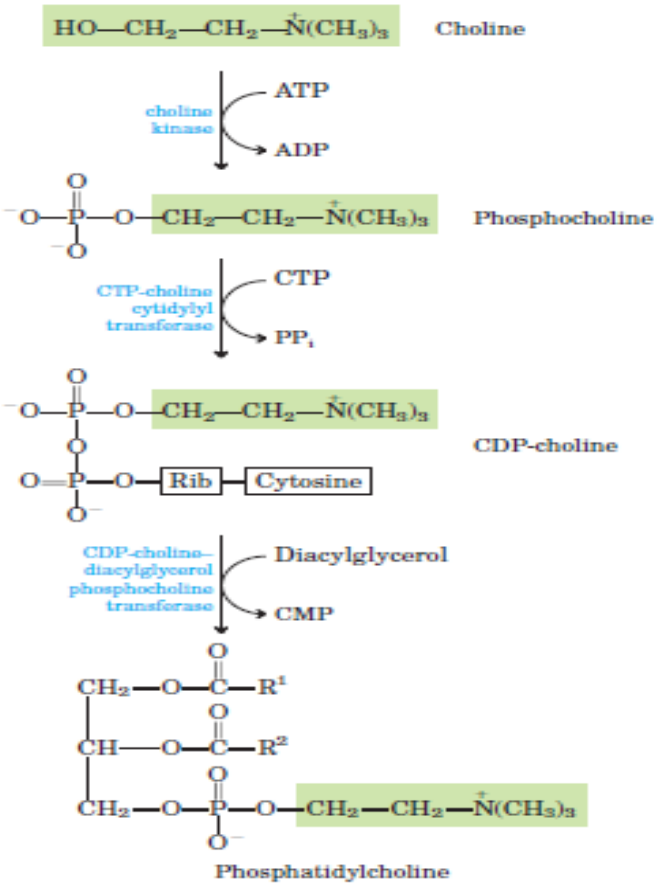
Specific **phosphatidylinositol kinases** then convert phosphatidylinositol to its phosphorylated derivatives. Phosphatidylinositol and its phosphorylated products in the plasma membrane play a central role in signal transduction in eukaryotes.

Eukaryotic Pathways to Phosphatidylserine, Phosphatidylethanolamine, and Phosphatidylcholine Are Interrelated

Yeast, like bacteria, can produce phosphatidylserine by condensation of CDP-diacylglycerol and serine, and can synthesize phosphatidylethanolamine from phosphatidylserine in the reaction catalyzed by phosphatidylserine decarboxylase. In mammalian cells, an alternative route to phosphatidylserine is a head-group exchange reaction, in which free serine displaces ethanolamine. Phosphatidylethanolamine may also be converted to **phosphatidylcholine** (lecithin) by the addition of three methyl groups to its amino group; *S*adenosylmethionine is the methyl group donor for all three methylation reactions. In mammals, phosphatidylserine is not synthesized from CDP-diacylglycerol; instead, it is derived from phosphatidylethanolamine via the head-group exchange reaction.



Choline is reused ("salvaged") by being phosphorylated then converted to CDP-choline by condensation with CTP. A diacylglycerol displaces CMP from CDP-choline, producing phosphatidylcholine.



An analogous salvage pathway converts ethanolamine obtained in the diet to phosphatidylethanolamine. In the liver, phosphatidylcholine is also produced by methylation of phosphatidylethanolamine (with *S*-adenosylmethionine, as described above), but in all other tissues phosphatidylcholine is produced only by condensation of diacylglycerol and CDP-choline. Although the role of lipid composition in membrane function is not entirely understood, changes in composition can produce dramatic effects. Researchers have isolated fruit flies with mutations in the gene that encodes ethanolamine kinase. Lack of this enzyme eliminates one pathway for phosphatidylethanolamine synthesis, thereby reducing the amount of this lipid in cellular membranes. Flies with this mutation—those with the genotype *easily shocked*—exhibit transient paralysis following electrical stimulation or mechanical shock that would not affect wild-type flies.

DEGRADATION OF PHOSPHOLIPIDS

Phospholipids are degraded by phospholipases which cleave the phosphodiester bonds. These enzymes are found in mammalian tissues, pancreatic juice, snake venom and in some toxins. Certain pathogenic bacteria produce phospholipases which help in the spread of infection by dissolving cell membranes.

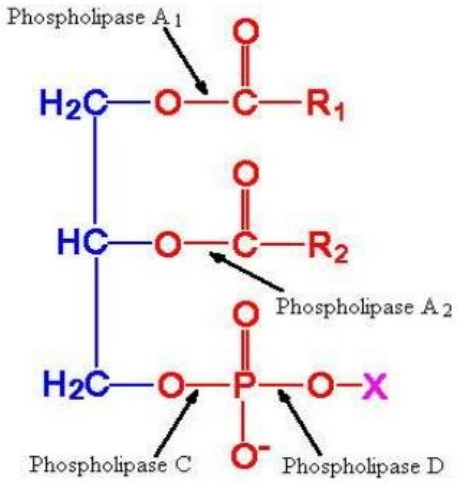
Phospholipase A_1 specifically cleaves the fatty acid at C_1 position of phospholipids resulting in lysophospholipid. The latter can be further acted by lysophospholipase, phospholipase B to remove the second acyl group at C_2 position.

Phospholipase A2 hydrolyses the fatty acid at C_1 position of phospholipids. Snake venom and bee venom are rich sources of phospholipase A2. This enzyme is found in many tissues and pancreatic juice. Phospholipase A2 acts on phosphatidyl inositol to liberate arachidonic acid, the substrate for the synthesis of prostaglandins.

Phospholipase C specifically cleaves the bond between phosphate and glycerol of phospholipids. This enzyme is present in lysosomes of hepatocytes. The toxins isolated from clostridia and other bacilli contain phospholipase C.

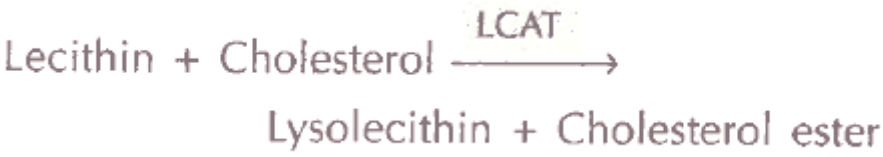
Phospholipase D hydrolyses and removes the nitrogenous base from phospholipids. This enzyme is mostly found in plant sources (cabbage, cotton, seed etc.). The degraded products of phospholipids enter the metabolic pool and are utilized for various purposes.

Degradation of phospholipids



Role of LCAT in lecithin metabolism

Lecithin-cholesterol acyl transferase (LCAT) is a plasma enzyme, synthesized in the liver. LCAT activity is associated with apo A1 of HDL. This enzyme esterifies cholesterol by transferring acyl group from the second position of lecithin



The above reaction is responsible for the reverse cholesterol transport mediated by HDL (more details given under lipoprotein metabolism

Degradation of sphingomyelins

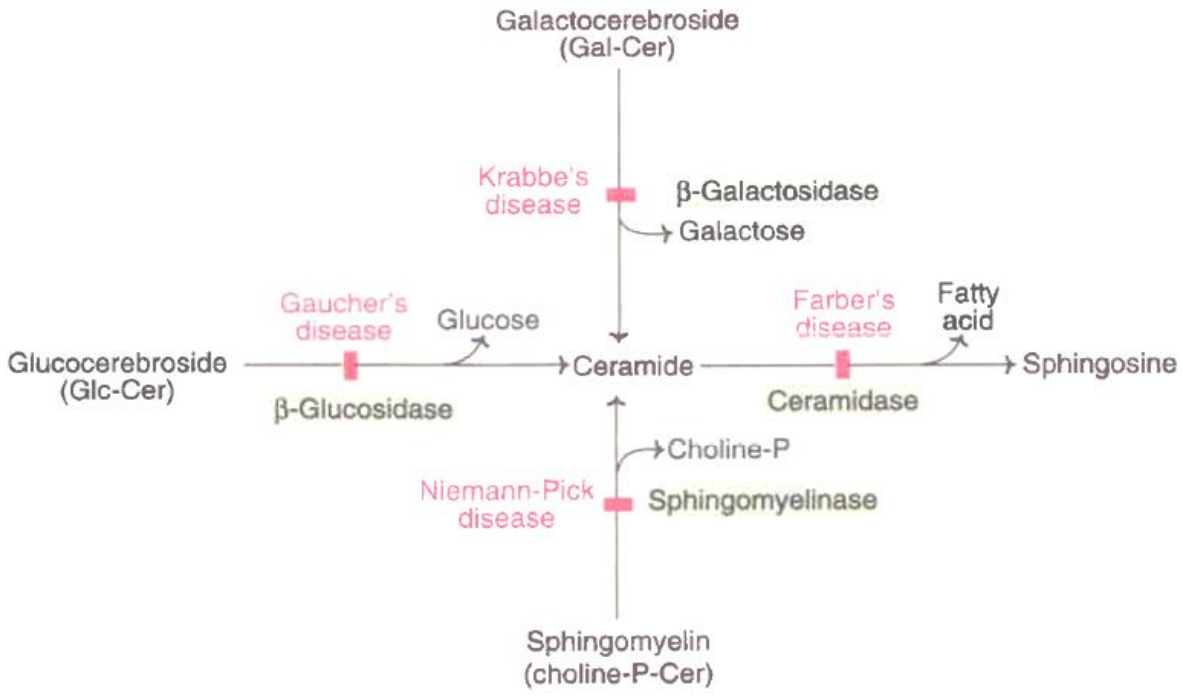
The enzyme sphingomyelinase of lysosomes hydrolyses sphingomyelins to ceramide and phosphorylcholine. Ceramide formed can be further degraded to sphingosine and free fatty acid.

Niemann-Pick disease: It is an inherited disorder due to a defect in the enzyme sphingomyelinase. This causes accumulation of sphingomyelins in liver and spleen, resulting in the enlargement of these organs. Victims of Niemann-Pick disease suffer from severe mental retardation, and death may occur in early childhood.

Farber's disease: A defect in the enzyme ceramidase results in Farber's disease. This disorder is characterized by skeletal deformation, subcutaneous nodules, dermatitis and mental retardation. It is fatal in early life"

BIOSYNTHESIS OF GLYCOLIPIDS

Glycolipids are derivatives of ceramide (sphingosine bound to fatty acid), hence they are more appropriately known as glycosphingolipids. The simplest form of glycosphingo lipids are cerebrosides containing ceramide bound to monosaccharides. Galactocerebroside (Gal-Cer) and glucocerebrosid (Glu-Cer) are the common glycosphingolipids Galactocerebrosidies a major component of membrane lipids in the nervous tissue (high in myelin sheath). Glucocerebroside is an intermediate in the synthesis and degradation of complex glycosphingo lipids.



Metabolic disorders of cerebrosides

Gaucher's disease: This is due to a defect in the enzyme β-glucosidase. As a result, tissue glucocerebroside levels increase. This disorder is commonly associated with enlargement of liver and spleen, osteoporosis, pigmentation of skin, anemia, mental retardation etc. Sometimes, Gaucher's disease is fatal.

Krabbe's disease: Defect in the enzyme β-galactosidase results in the accumulation of galactocerebrosides. A total absence of myelin in the nervous tissue is a common feature. Severe mental retardation, convulsions, blindness, deafness etc. are seen. Krabbe's disease is fatal in early life.

Gangliosides are complex glycosphingolipids mostly found in ganglion cells. They contain one or more molecules of N-acetylneuraminic acid (NANA) bound ceramide ligosaccharides Defect in the degradation of gangliosides causes gangliosidosis, Tay-Sach's disease etc.

Sphinognlipidoses: Lipid storage diseases, representing Iysosomal storage defects, are inherited disorders. They are characterized by the accumulation of complex lipids. The term sphingolipidoses is often used to collectively refer to the genetic disorders that lead to the accumulation of any one of the sphingolipids (glycosphingolipids and sphingomyelins).

METABOLISM OF CHOLESTEROL

Cholesterol is an essential molecule in many animals, including humans, but is not required in the mammalian diet- all cells can synthesize it from simple precursors. It is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids, and vitamin D. As a typical product of animal metabolism, cholesterol occurs in foods of animal origin such as egg yolk, meat, liver, and brain.

The isoprene units that are the essential intermediates in the pathway from acetate to cholesterol are also precursors to many other natural lipids and the mechanisms by which isoprene units are polymerized are similar in all these pathways.

Cholesterol Is Made from Acetyl-CoA in Four Stages

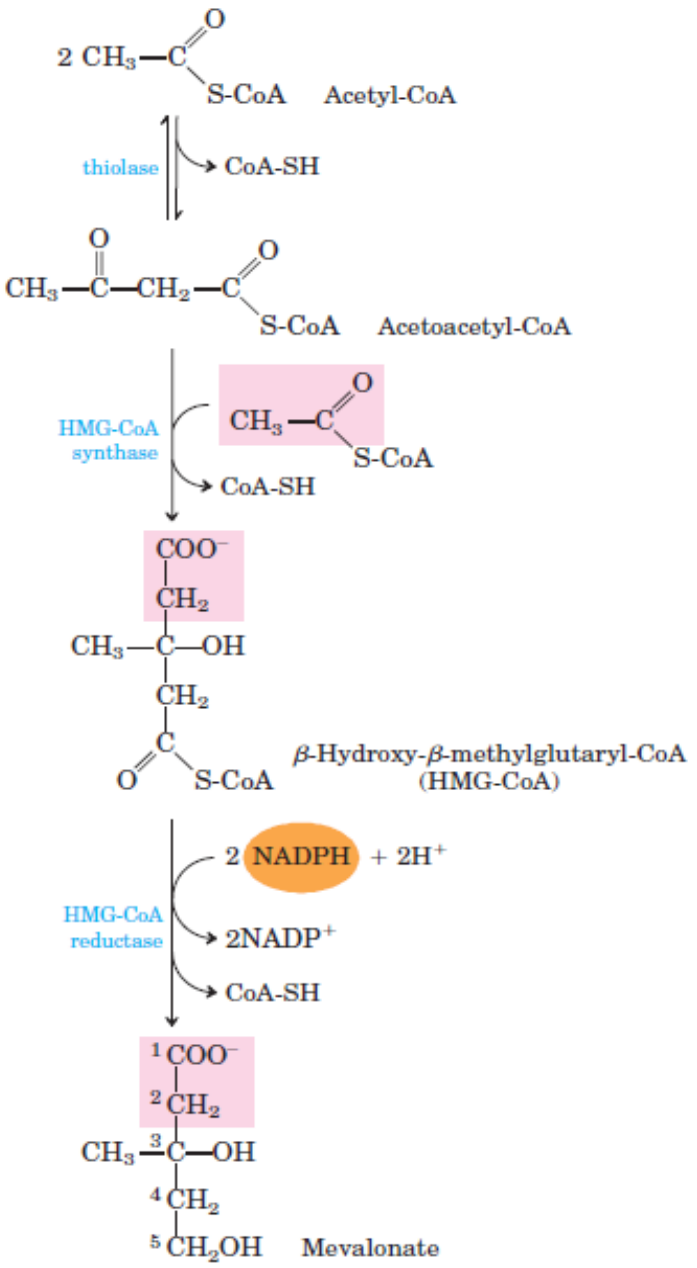
Cholesterol, like long-chain fatty acids, is made from acetyl-CoA, but the assembly plan is quite different. In early experiments, animals were fed acetate labelled with 14C in either the methyl carbon or the carboxyl carbon. Synthesis takes place in four stages, 1 condensation of three acetate units to form a six-carbon intermediate, mevalonate; 2 conversion of mevalonate to activated isoprene units; 3 polymerization of six 5-carbon isoprene units to form the 30-carbon linear squalene; and 4 cyclization of squalene to form the four rings of the steroid nucleus, with a further series of changes (oxidations, removal or migration of methyl groups) to produce cholesterol. The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by NADPH while ATP provides energy. For the production of one mole of cholesterol, 18 moles of acetyl-CoA, 36 moles of ATP and 16 moles of NADPH are required.

Stage 1 Synthesis of Mevalonate from Acetate

The first stage in cholesterol biosynthesis leads to the intermediate mevalonate. Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, which condenses with a third molecule of acetyl-CoA to yield the six-carbon compound β-hydroxy- β -methylglutaryl-CoA (HMG-CoA). These first two reactions are catalyzed by thiolase and HMG-CoA synthase, respectively. The cytosolic HMG-CoA synthase in this pathway is distinct from the mitochondrial isozyme that catalyzes HMG-CoA synthesis in ketone body formation. The third

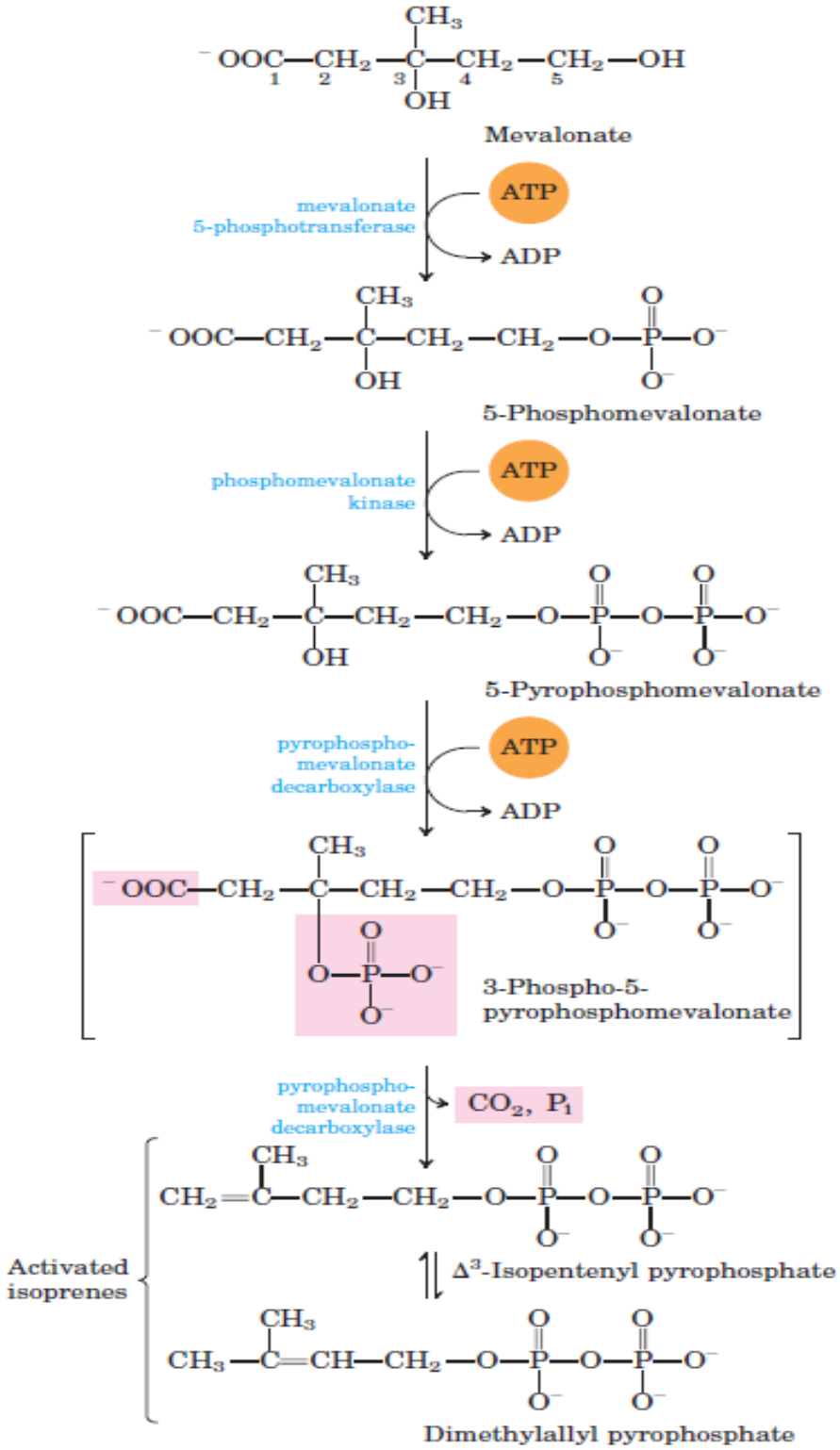
reaction is the committed and rate-limiting step: reduction of HMG-CoA to mevalonate, for which each of two molecules of NADPH donates two electrons. **HMG-CoA reductase,** an integral membrane protein of the smooth ER, is the major point of regulation on the pathway to cholesterol.

Formation of mevalonate from acetyl-CoA: The origin of C-1 and C-2 of mevalonate from acetyl-CoA is shown in pink.



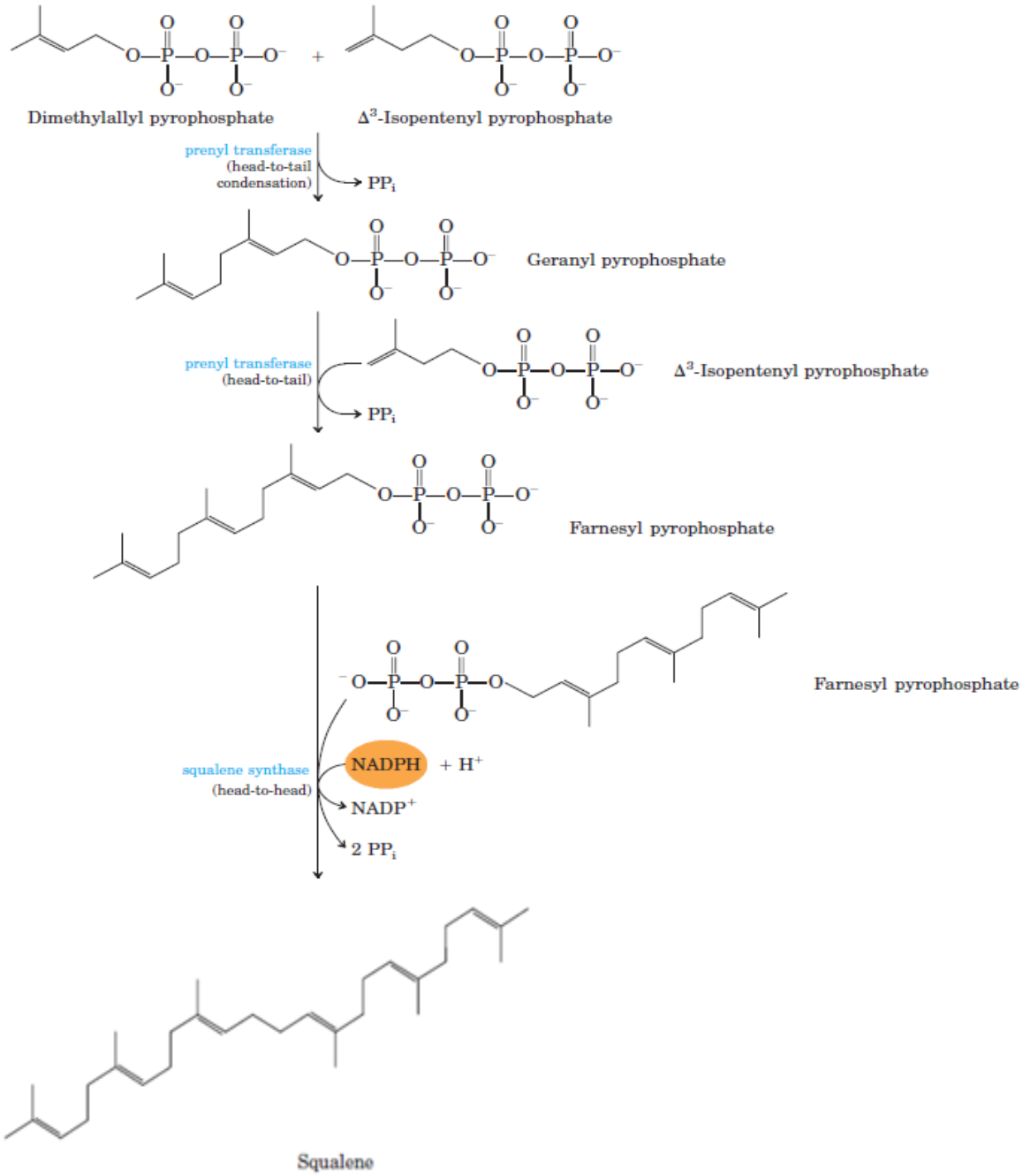
Stage 2 Conversion of Mevalonate to Two Activated Isoprenes

In the next stage of cholesterol synthesis, three phosphate groups are transferred from three ATP molecules to mevalonate. The phosphate attached to the C-3 hydroxyl group of mevalonate in the intermediate 3-phospho-5-pyrophosphomevalonate is a good leaving group; in the next step, both this phosphate and the nearby carboxyl group leave, producing a double bond in the five-carbon product, Δ^3**-isopentenyl pyrophosphate.** This is the first of the two activated isoprenes central to cholesterol formation. Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, **dimethylallyl pyrophosphate.**



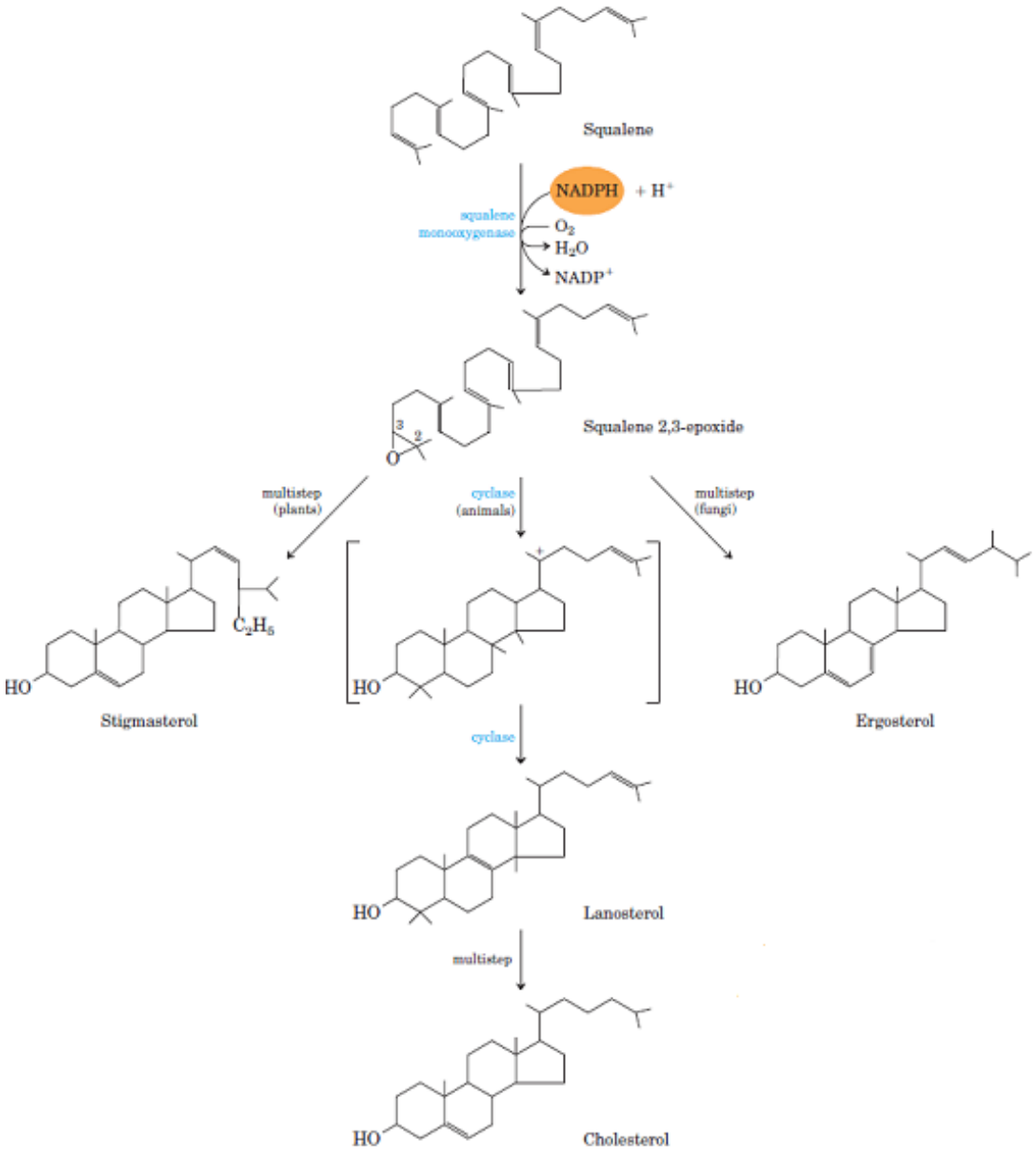
Stage 3 Condensations of Six Activated Isoprene Units to Form Squalene

Isopentenyl pyrophosphate and dimethylallyl pyrophosphate now undergo a head-to-tail condensation, in which one pyrophosphate group is displaced and a 10-carbon chain, **geranyl pyrophosphate,** is formed. Geranyl pyrophosphate undergoes another head-to-tail condensation with isopentenyl pyro-phosphate, yielding the 15-carbon intermediate **farnesyl pyrophosphate.** Finally, two molecules of farnesyl pyrophosphate join head to head, with the elimination of both pyrophosphate groups, to form **squalene.**



Stage 4 Conversion of Squalene to the Four-Ring Steroid Nucleus

When the squalene molecule is represented the relationship of its linear structure to the cyclic structure of the sterols becomes apparent.



Ring closure converts linear squalene to the condensed steroid Nucleus: The first step in this sequence is catalyzed by a mixed-function oxidase (a monooxygenase), for which the cosubstrate is NADPH. The product is an epoxide, which in the next step is cyclized to the steroid nucleus. The final product of these reactions in animal cells is cholesterol; in other organisms, slightly different sterols are produced.

The action of squalene monooxygenase adds one oxygen atom from O_2 to the end of the squalene chain, forming an epoxide. This enzyme is another mixed-function oxidase NADPH reduces the other oxygen atom of O_2 to H_2O. The double bonds of the product, squalene 2,3-epoxide, are positioned so that a remarkable concerted reaction can convert the linear squalene epoxide to a cyclic structure. In animal cells, this cyclization results in the formation of lanosterol, which contains the four rings characteristic of the steroid nucleus. Lanosterol is finally converted to cholesterol in a series of about 20 reactions that include the migration of some methyl groups and the removal of others. Cholesterol is the sterol characteristic of animal cells; plants, fungi and protists make other, closely related sterols instead. They use the same synthetic pathway as far as squalene 2, 3-epoxide, at which point the pathways diverge slightly, yielding other sterols, such as stigmasterol in many plants and ergosterol in fungi. Regulation of cholesterol synthesis is exerted near the beginning of the pathway, at the HMG-CoA reductase step. The reduced synthesis of cholesterol in starving animals is accompanied by a decrease in the activity of the enzyme. However, it is only hepatic synthesis that is inhibited by dietary cholesterol. HMG-CoA reductase in liver is inhibited by mevalonate, the immediate product of the pathway, and by cholesterol, the main product.

Summary

In the first stage of β -oxidation, four reactions remove each acetyl-CoA unit from the carboxyl end of a saturated fatty acyl–CoA: (1) dehydrogenation of the α and β carbons (C-2 and C-3) by FAD-linked acyl-CoA dehydrogenases, (2) hydration of the resulting ***trans***-Δ^2 double bond by enoyl-CoA hydratase, (3) dehydrogenation of the resulting L- β hydroxyacyl-CoA by NAD-linked β -hydroxyacyl-CoA dehydrogenase, and (4) CoA-requiring cleavage of the resulting β -ketoacyl-CoA by thiolase, to form acetyl-CoA and a fatty acyl–CoA shortened by two carbons. The shortened fatty acyl–CoA then re enters the sequence.

In the second stage of fatty acid oxidation, the acetyl-CoA is oxidized to CO_2 in the citric acid cycle. A large fraction of the theoretical yield of free energy from fatty acid oxidation is recovered as ATP by oxidative phosphorylation, the final stage of the oxidative pathway. Malonyl-CoA, an early intermediate of fatty acid synthesis, inhibits carnitine acyltransferase I, preventing fatty acid entry into mitochondria. This blocks fatty acid breakdown while synthesis is occurring.

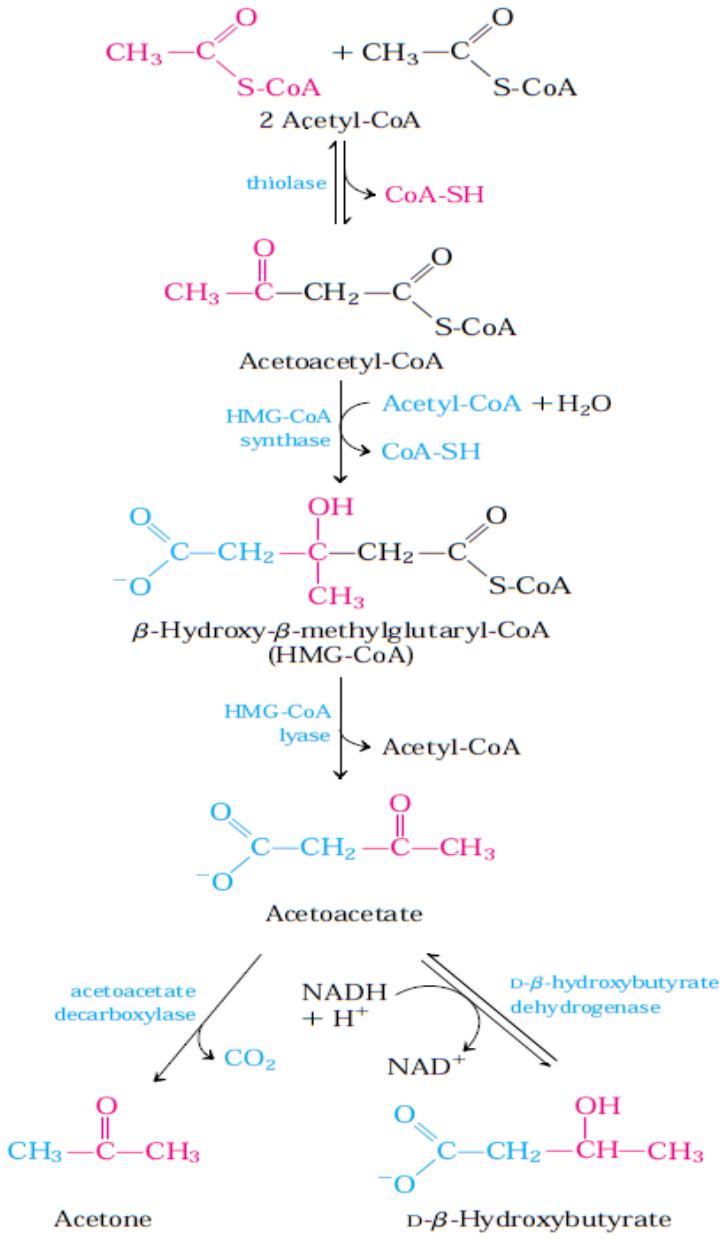
Cholesterol is formed from acetyl-CoA in a complex series of reactions, through the intermediates β -hydroxy- β -methylglutaryl-CoA, mevalonate and two activated isoprenes, dimethylallyl pyrophosphate and isopentenyl pyrophosphate. Condensation of isoprene units produces the noncyclic squalene, which is cyclized to yield the steroid ring system and side chain. Cholesterol synthesis is under hormonal control and is also inhibited by elevated concentrations of intracellular cholesterol, which acts through covalent modification and transcriptional regulation mechanisms.

KETONE BODIES

In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the "ketone bodies," acetone, acetoacetate, and D-β-hydroxybutyrate, for export to other tissues. Acetone, produced in smaller quantities than the other ketone bodies, is exhaled. Acetoacetate and D-β-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex. The brain, which preferentially uses glucose as fuel, can adapt to the use of acetoacetate or D-β-hydroxybutyrate under starvation conditions, when glucose is unavailable. The production and export of ketone bodies from the liver to extrahepatic tissues allow continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle.

Ketone Bodies, Formed in the Liver, Are Exported to Other Organs as Fuel

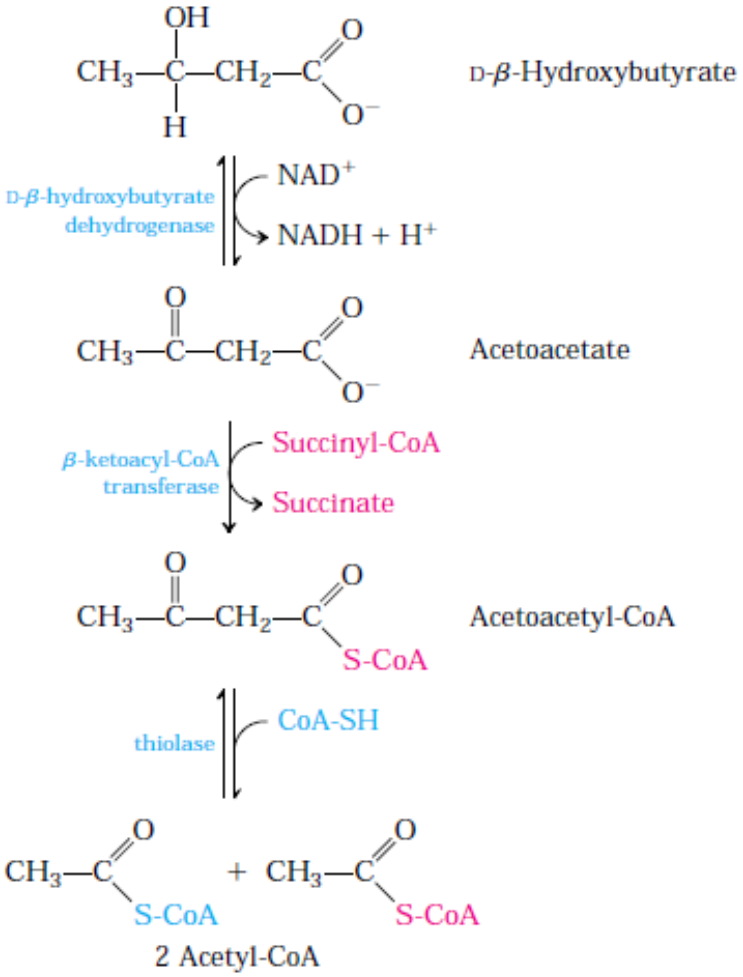
The first step in the formation of acetoacetate, occurring in the liver, is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase; this is simply the reversal of the last step of β-oxidation. The acetoacetyl-CoA then condenses with acetyl-CoA to form β-**hydroxy**-β-**methylglutaryl-CoA (HMG-CoA),** which is cleaved to free acetoacetate and acetyl-CoA. The acetoacetate is reversibly reduced by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-β-hydroxybutyrate. This enzyme is specific for the D stereoisomer; it does not act on L-β-hydroxyacyl-CoAs and is not to be confused with L-β-hydroxyacyl-CoA dehydrogenase of the β-oxidation pathway.



In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of **acetoacetate decarboxylase.** Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic. Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.

In extrahepatic tissues, D-β-hydroxybutyrate is oxidized to acetoacetate by D-β-hydroxybutyrate dehydrogenase. The acetoacetate is activated to its coenzyme A ester by

transfer of CoA from succinyl- CoA, an intermediate of the citric acid cycle, in a reaction catalyzed by β-**ketoacyl-CoA transferase.** The acetoacetyl-CoA is then cleaved by thiolase to yield two acetyl-CoAs, which enter the citric acid cycle. Thus the ketone bodies are used as fuels. The production and export of ketone bodies by the liver allow continued oxidation of fatty acids with only minimal oxidation of acetyl-CoA. When intermediates of the citric acid cycle are being siphoned off for glucose synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slows and so does acetyl-CoA oxidation. Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl- CoA, β-oxidation slows for want of the free coenzyme. The production and export of ketone bodies free coenzyme A, allowing continued fatty acid oxidation.



Ketone Bodies Are Overproduced in Diabetes and during Starvation

Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid

cycle intermediates, diverting acetyl-CoA to ketone body production. In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat. Under these conditions, levels of malonyl- CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to acetyl- CoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis. The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them. The increased blood levels of acetoacetate and D-β-hydroxybutyrate lower the blood pH, causing the condition known as **acidosis.** Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of untreated diabetics can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of < 3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of ≤ 125 mg/ 24 hr). This condition is called **ketosis.** Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis).

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE - 21
DEPARTMENT OF BIOCHEMISTRY
III B.Sc BIOCHEMISTRY
BATCH: 2015 - 2018

SUBJECT: METABOLIC PATHWAYS **SUBJECT CODE: 15BCU501** **UNIT: III**

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

S. No	Question	Option A	Option B	Option C	Option D	Answer
	Which enzyme is involved in lipid digesion?	Elastase	lactase	Lipase	Lactate dehydrogenase	Lipase
	Digesion of triglycerides requires _____	Bile salts	Bile pigments	Intrinsic factor	Bile acids	Bile salts
	Absorption of fats occurs mainly in_____	Stomach	Duodenum	Jejunum	Ileum	Jejunum
	Fatty acids are degraded mainly by_____	ω-oxidation	α- oxidation	β-oxidation	HMP shunt	β-oxidation
	Majority of the absorbed fat appears in the form of_____	VLDL	LDL	HDL	Chylomicrones	Chylomicrones
	The end product of fatty acid synthesis in mammals is_____	Arachidonic acid	Linoleic acid	Stearic acid	Palmitic acid	Palmitic acid
	The key regulatory enzyme of fatty acid synthesis is _____	Acyl coA synthetase	Acetyl coA carboxylase	Keto acyl synthase	Thioesterase	Acetyl coA carboxylase
	NADPH required for fatty acid synthesis can be generated from _____	HMP shunt	Glycolysis	TCA cycle	Urea cycle	HMP shunt
	Which of the following inhibits the acetylCoA carboxylase a rate limiting enzymes of carbohydrate metabolism?	Citrate	ATP	Malonyl CoA	Acyl CoA	Acyl CoA
	Malonyl-CoA A is a direct inhibitor of which enzyme of fatty acid oxidation_____	**Carnitine Acyl Transferase –I**	**Carnitine Acyl Transferase –II**	**Thiokinase**	**Acyl co A synthetase**	**Carnitine Acyl Transferase –I**
	β-oxidation of long chain fatty acids occurs primarily in _____	Cytosol	Peroxisomes	Mitochondria	Golgi apparatus	Mitochondria
	Cholestrol is the precursor of_____	Steroid hormones	Vitamin A	Urea	Folic acid	Steroid hormones
	The committed step in cholesterol biosynthesis	Formation of squaline	Formation of HMG CoA	Formation of mevalonic acid from HMG CoA	Cyclisation of squaline to lanodterol	Formation of mevalonic acid from HMG CoA
	The principle building block of fatty acid is _____	Succinyl CoA	Acetyl CoA	Propionyl CoA	Acetoacetyl CoA	Acetyl CoA
	Biosynthesis of fatty acid requires which vitamin?	Riboflavin	Pyridoxine	Thiamin	Pantothenic acid	Pantothenic acid
	ACP is involved in the synthesis of _____	Phospholipids	Fatty acids	Glycogen	Triglycerides	Fatty acids
	The main catabolic end product of cholesterol is _____	Acetyl CoA	Propionyl CoA	Coprosterol	Bile acids	Bile acids
	The fattyacid synthase complex comprises two monomers, each containing_____	2 enzymes	5 enzymes	7 enzymes	10 enzymes	7 enzymes
	β-oxidation of palmitic acid produces a net synthesis of how many ATP molecules?	109	129	24	38	129
	Bile acid are derived from_____	Cholesterol	Amino acids	Fatty acids	Bilirubin	Cholesterol
	The major storage form of lipids is_____	Esterified cholesterol	Glycerophospholipids	Triglycerides	Sphingolipids	Triglycerides
	The principal precursors of glycerophospholipids are	Phospholipids	Spingolipids	Diacylglycerols	Spingomyelins	Diacylglycerols
	The important lipid involved in cell adhesion and cell recognition is	Phospholipids	Cholesterol	Glycospingolipids	Ceramide	Glycospingolipids
	Acyl Carrier Protein contains the vitamin_____	Biotin	Lipoic acid	Pantothenic acid	Folic acid	Pantothenic acid
	α-Oxidation of fatty acids occurs mainly in_____	Liver	Brain	Muscles	Adipose tissue	Brain
	The enzyme involved ω-oxidation are located in	golgi complex	Cytoplasm	Endoplasmic recticulum	Mitochondria	Endoplasmic recticulum
	The rate of fatty acid oxidation is increased by _____	Phospholipids	Glycolipids	Amino lipids	Spingolipids	Phospholipids
	The starting material for the process of ketogenesis is_____	Acetyl CoA	Oxaloacetate	Pyruvate	Citrate	Acetyl CoA
	Which among the following is the most complex sphingolipid_____	Cerebroside	Gangleoside	Globoside	Ceramide	Gangleoside
	How many double bonds occur in Arachidonic acid_____	1	2	3	4	4
	Which of the following is essential fatty acid_____	Linolenic acid	Arachidonic acid	Oleic acid	Palmitic acid	Linolenic acid
	A genetic disorder caused by the accumulation of sphingomyelin in brain is called_____	Tay-Sach syndrome	Gout	Niemann-Pick Disease	Gauche's disease	Niemann-Pick Disease
	Lipid molecule involved in the bio-signaling pathway that include membrane turnover and exocytosis is _____.	Phosphatidylinositol	Phosphatidyl glycerol	Myoinositol	Phosphatidyl glycerol and Myoinositol	Phosphatidylinositol
	Most abundant membrane lipid in the biosphere is_____	**Phospholipid**	**Galactolipid**	**Sphingolipid**	**Ether lipid**	**Galactolipid**
	What is the molecular formula of cholesterol?	$C_{27}H_{45}OH$	$C_{28}H_{47}OH$	$C_{29}H_{47}OH$	$C_{23}H_{41}OH$	$C_{27}H_{45}OH$
	Enzymes for beta oxidation of fatty acids are located in_____	Mitochondria	Mitochondria and cytoplasm	Mitochondria and Golgi	Mitochondria and peroxisome	Mitochondria
	Cerebroside may also classified as____	Phospholipid	Sphingolipid	Aminolipid	Glycolipid	Sphingolipid
	Glyco-sphingolipids are a combination of_____	Glycerol with two galactose residues	Ceramide with one or more sugar residues	Sphingosine with galactose and ceramide	Sphingosine with glucose	Ceramide with one or more sugar residues
	Spingomyelins contain a complex amino alcohol named as_____	Serine	Lysolecithin	Spingosine	Glycol	Spingosine
	The key regulatory enzyme of cholesterol synthesis is_____	HMG- Co A synthase	HMG Co A lyase	HMG Co A reductase	Mevalonate kinase	HMG Co A reductase
	The enzyme 'Thiolase' catalyzes the conversion of_____	2 Acetyl co A to Acetoacetyl co A	Acetyl co A to Malonyl co A	Fatty acid to Fatty Acyl co A	Succinyl co A to succinate	2 Acetyl co A to Acetoacetyl co A
	The enzyme involved in mammalian signal transduction is	Phospholipase A	Phospholipase B	Phospholipase C	Phospholipase D	Phospholipase D
	In alpha oxidation which of the following products is released ?	Co A	CO_2	H_2O	Acetyl co A	CO_2
	Which of the following is a break down product of odd chain fatty acids?	Acetyl co A only	Acetyl co A and Butyryl co A	Acetyl co A and Propionyl co A	Malonyl co A	Acetyl co A and Propionyl co A
	All the 27 carbon atoms of cholesterol are derived from_____	Acetyl co A	Acetoacetyl co A	Propionyl co A	Succinyl co A	Acetyl co A
	NADPH is synthesized by the action of which of the following enzymes?	Glucose-6-P dehydrogenase	Pyruvate dehydrogenase	Acetyl co A carboxylase	Lipoprotein lipase	Glucose-6-P dehydrogenase
	How many carbons are removed from fatty acyl co A in one turn of β- oxidation spiral ?	1	2	3	4	2
	What is the role of Thiolase in the β- oxidation of fatty acids?	Cleaves of Co A	Cleaves the bond between α- and β- carbons	Adds H_2O across the double bond	Generates NADH	Cleaves the bond between α- and β- carbons
	The key enzyme for the utilization of ketone bodies is_____	Thiolase	Thiophorase	Thiokinase	Thioesterase	Thiophorase
	High content of triglycerides are seen in_____	LDL	HDL	VLDL	Chylomicrones	Chylomicrones
	Gangliosides are glycolipids occurring in_____	Liver	Brain	Kidney	Muscle	Brain
	The prostaglandins are synthesized from _____	Aracadonic acid	Oleic acid	Linoleic acid	Linolenic acid	Aracadonic acid
	Prostaglandins are liberated in the circulation by the stimulation of _____	Anterior pitutary glands	Posterior pitutary glands	Adrenal gland	Thyroid gland	Adrenal gland
	The synthesis of prostaglandins is inhibited by_____	Aspirin	Arsenite	Fluoride	Cyanide	Aspirin
	HDL is synthesized and secreted from_____	Pancrease	Liver	Kidney	Muscle	Liver
	Fatty liver caused by_____	CH_2Cl	CCL_4	$MgSO_4$	CH_3COOH	CCL_4
	Ketosis generally occurs in_____	Nephritis	Oedema	Infective hepatic disease	Coronary thrombosis	Infective hepatic disease
	Ketone bodies are utilized in _____	Mitochondria	Extrahepatic tissues	Nuclei	Chromosomes	Extrahepatic tissues
	Eicosanoids are formed from _____	Arachidonate	Palmitate	Stearate	Butyrate	Arachidonate
	The excretion of ketone bodies in the urine involves the deficiency of _____	Na^+	Fe^{++}	Ca^{++}	Mg^{++}	Na^+

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(For the candidates admitted from 2015 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT : METABOLIC PATHWAYS
SEMESTER : V
SUBJECT CODE : 15BCU501 CLASS : III B.Sc.BC

UNIT IV-- COURSE MATERIAL

Metabolism of protein and amino acids

Introduction, fate of dietary proteins, catabolism of aminoacid nitrogen-oxidative deamination; non-oxidative deamination, transamination-formation of ammonia, transport of ammonia, disposal of ammonia -urea cycle. Amino acid decarboxylation, Catabolism of carbon skeleton of aminoacids- glycine, tyrosine, phenyl alanine, glutamic acid and lysine.

TEXT BOOKS

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Pamela C, Champ Richard and A. Harvey.2008. Biochemistry. Lipponcott Company Piladelphia.

UNIT IV

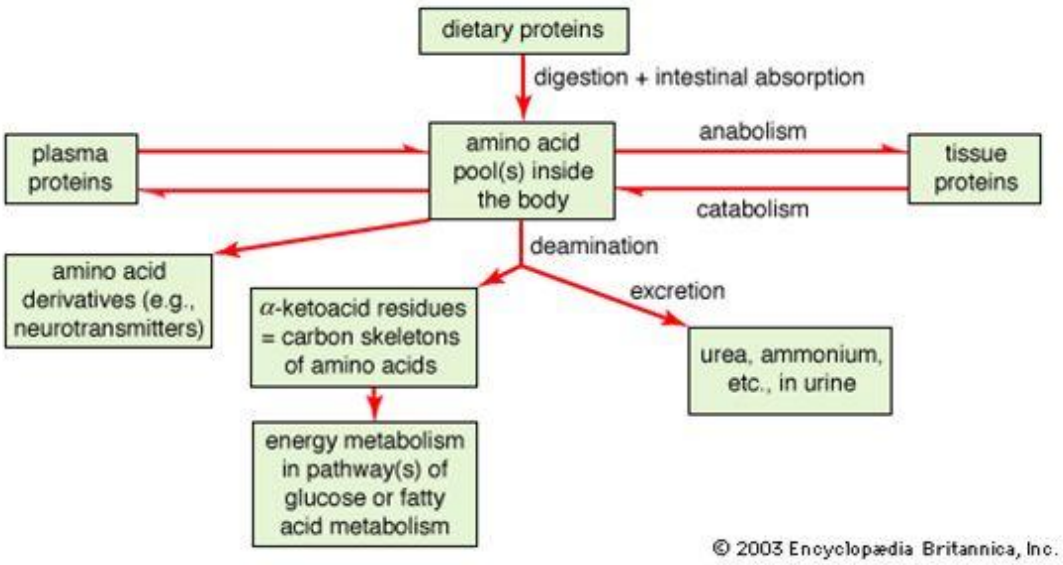
METABOLISM OF PROTEIN AND AMINO ACIDS

Introduction

Proteins are the most abundant organic compounds and constitute a major part of the body dry weight (10-12 kg in adults). All body proteins continuously undergo degradation and synthesis. More than half of the protein of the liver and intestinal mucosa are broken down and re synthesised once in ten days. The rate is slower in muscles and erythrocytes. The proteins on degradation release individual amino acids. Protein metabolism is more appropriately learnt as metabolism of amino acids. Amino acids contain nitrogen in addition to the carbon, hydrogen and oxygen atoms also found in carbohydrates and fats.

Fate of Dietary proteins

The human body usually needs more than just protein to maintain appropriate nitrogen levels. Dietary protein should be of high quality, such as that typically found in eggs and lean meats. The human body also generally needs an adequate supply of fats and carbohydrates. These macronutrients are widely considered essential to energy production. When dietary levels of fat and carbohydrate fall too low, the body uses protein for energy, which may deplete the body's nitrogen levels and inhibit the body's ability to renew damaged cells.



Metabolism of Dietary proteins

Catabolism of amino acid nitrogen

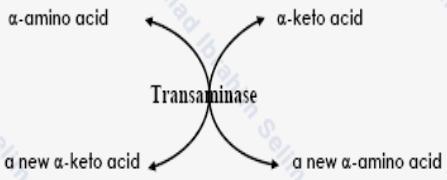
- In mammalian tissues, the α-amino group of amino acids derived from the diet or from the tissue protein breakdown is ultimately excreted in urine as urea. Several enzymes are involved in the process. Biosynthesis of urea involves four processes:
- Transamination
- Oxidative deamination of glutamate.
- Ammonia transport
- Reactions of urea cycle.

Catabolic pathways of amino acids

1. Transamination
2. Deamination
3. Transamidination
4. Transamidation
5. Decarboxylation

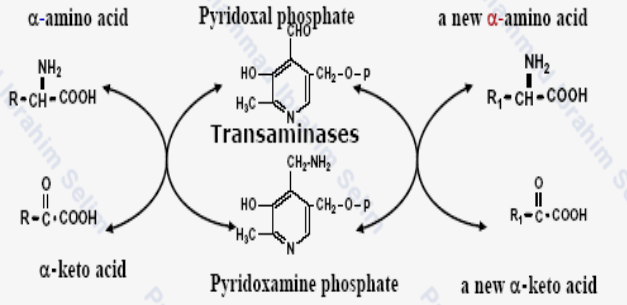
1- Transamination

Transamination means transfer of amino group from α-amino acid to α-keto acid with formation of a new α-amino acid and a new α-keto acid.
The liver is the main site for transamination.
All amino acids can be transaminated except lysine, threonine, proline and hydroxy proline.
All transamination reactions are reversible.
It is catalyzed by aminotransferases (transaminases).
It needs pyridoxal phosphate as a coenzyme.



Role of pyridoxal phosphate in transamination

Pyridoxal phosphate acts as an intermediate carrier for amino group
Pyridoxal phosphate accepts the amino group from amino acid to form pyridoxamine phosphate, which in turn gives the amino group to α-keto acid

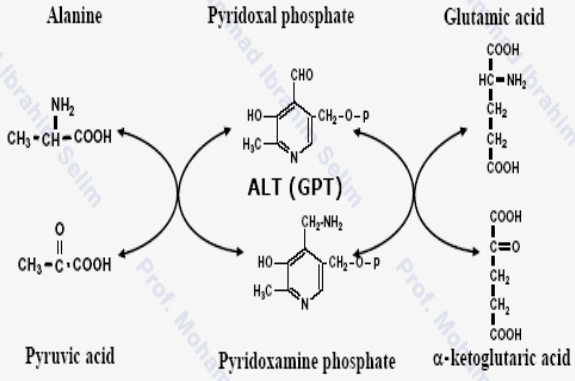


Examples of transaminases

A. Alanine transaminase
B. Aspartate transaminase
C. Glutamate transaminase

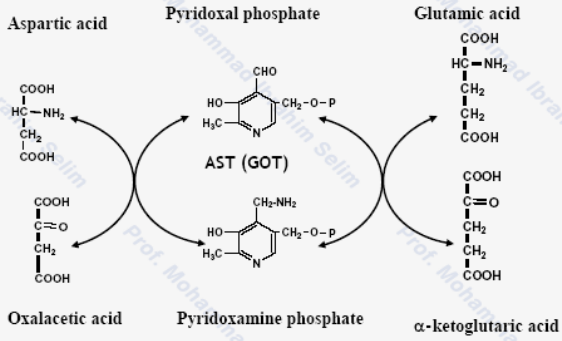
A. Alanine transaminase (ALT)

- It is also called glutamic pyruvic transaminase (GPT).
- It catalyzes the transfer of amino group from glutamic acid to pyruvic acid to form alanine and α-ketoglutaric acid.
- It also catalyzes the reverse reaction.
- It needs pyridoxal phosphate as a coenzyme.
- It is present in the cytoplasm of liver cells.



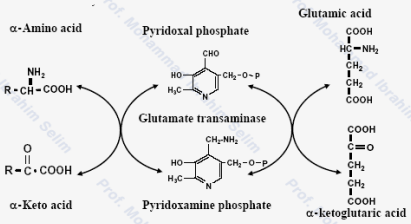
B. Aspartate transaminase (AST)

- It is also called glutamic oxalacetic transaminase (GOT)
- It catalyzes the transfer of amino group from glutamic acid to oxalacetic acid to form aspartic acid and α-ketoglutaric acid
- It also catalyzes the reverse reaction
- It needs pyridoxal phosphate as a coenzyme
- It is present in liver, heart and skeletal muscle cells.
- It is present in both cytoplasm and mitochondria



C. Glutamate transaminase

- It catalyzes the transfer of amino group from any amino acid (except lysine, threonine, proline and hydroxy proline) to α-ketoglutaric acid to form glutamic acid and the corresponding α-keto acid
- It also catalyzes the reverse reaction
- It needs pyridoxal phosphate as a coenzyme
- It is widely distributed in all tissues



Clinical significance of serum transaminases

Transaminases are intracellular enzymes.
Their levels in blood plasma are low under normal conditions.
ALT (GPT) is present mainly in the cytoplasm of liver cells.
AST (GOT) is present in both cytoplasm and mitochondria in liver, heart and skeletal muscles.
Any damage to these organs will increase the level of transaminases in blood
In liver diseases, there is an increase in both serum ALT (SGPT) and AST (SGOT) levels.
In acute liver diseases, e.g. acute viral hepatitis, the increase is more in SGPT
In chronic liver diseases, e.g. liver cirrhosis the increase is more in SGOT.
In heart diseases, e.g. myocardial infarction, there is an increase in SGOT only.
In skeletal muscle diseases, e.g. myasthenia gravis, there is an increase in SGOT only.

Deamination

Deamination means the removal of amino group from α-amino acid in the form of ammonia with formation of α-keto acid
The liver and kidney are the main sites for deamination

Deamination may be oxidative or non-oxidative

A. Oxidative deamination
It is catalyzed by one of the following enzymes:

1. L-amino acid oxidases
2. D-amino acid oxidases
3. Glutamate dehydrogenase

B. Non-oxidative deamination
It is catalyzed by one of the following enzymes:

1. Dehydratases
2. Desulfhydrases

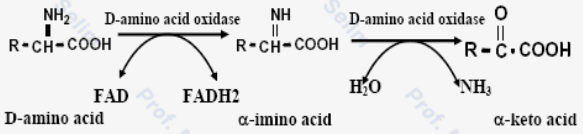
A. Oxidative deamination

1- L amino acid oxidase

- This enzyme is present in the liver and kidney. Its activity is low.
- It is an aerobic dehydrogenase that needs FMN as a coenzyme.
- It deaminates most of the naturally occurring L-amino acids

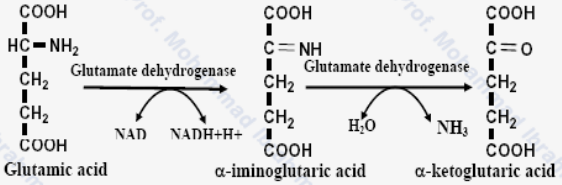
2- D amino acid oxidase

- D- amino acids are present in plants and bacterial cell wall.
- They are not used in protein biosynthesis in humans and animals.
- D-amino acids are deaminated by D-amino acid oxidase resulting in ammonia and α-keto acids.
- D-amino acid oxidase is present in the liver.
- It is an aerobic dehydrogenase.
- It needs FAD as a coenzyme.



3- Glutamate dehydrogenase

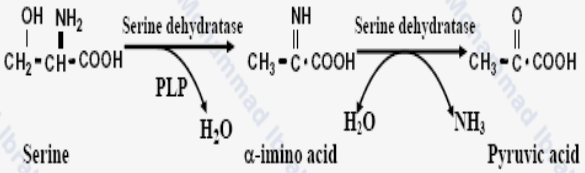
- This enzyme is present in most tissues
- It is present both in cytoplasm and mitochondria
- Its activity is high
- It is an anaerobic dehydrogenase
- It needs NAD or NADP as a coenzyme
- It deaminates glutamic acid resulting in α-ketoglutaric acid and ammonia



B. Non-oxidative deamination

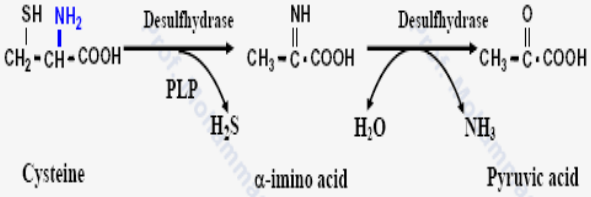
1- Dehydratase

This enzyme deaminates amino acids containing hydroxyl group e.g. serine, homoserine and threonine.
It needs pyridoxal phosphate as coenzyme.



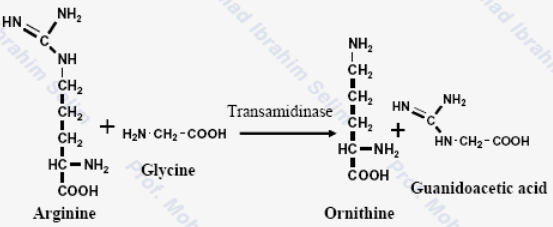
2-Desulfhydrase

This enzyme deaminates sulpher containing amino aids e.g. cysteine and cystine.
It needs pyridoxal phosphate as a coenzyme.



Transaminidation

Transamidination means the transfer of amidine group from a donor molecule to an acceptor molecule
It is catalyzed by transamidinase enzyme
An example of transmidination reaction is the transfer of amidine group from arginine (donor) to glycine (acceptor) in creatine biosynthesis



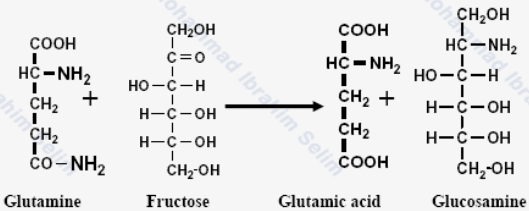
Transamidation

Transamidation means transfer of amide group nitrogen from a donor molecule to an acceptor molecule
It is catalyzed by transamidase enzyme

Examples of transmidation reaction include:

1. Transfer of amide nitrogen from glutamine (donor) to fructose (acceptor) to form glucosamine
2. Amide group nitrogen of glutamine is the source of N3 and N9 in purine bases

Glucosamine biosynthesis

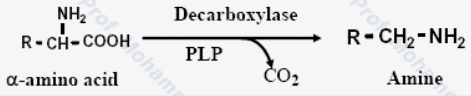


Decarboxylation

Decarboxylation means removal of CO2 from amino acid with formation of corresponding amines
It is catalyzed by decarboxylase enzyme
It needs pyridoxal phosphate as a coenzyme

Examples of decarboxylation reaction include:

1. Decarboxylation of histidine to form histamine
2. Decarboxylation of tyrosine to form tyramine



Amino acid metabolism

The amino acids not only function as energy metabolites but also used as precursors of many physiologically important compounds such as heme, bioactive amines, small peptides, nucleotides and nucleotide coenzymes.

In normal human beings about 90% of the energy requirement is met by oxidation of carbohydrates and fats. The remaining 10% comes from oxidation of the carbon skeleton of

amino acids. Since the 20 common protein amino acids are distinctive in terms of their carbon skeletons, amino acids require a unique degradative pathway. The degradation of the carbon skeletons of 20 amino acids converges to just seven metabolic intermediates namely,

i. Pyruvate ii. Acetyl CoA iii. Acetoacetyl CoA iv. -α-Ketoglutarate v. Succinyl CoA vi. Fumarate vii. Oxaloacetate -ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as α Pyruvate, precursors for glucose synthesis through gluconeogenesis. Amino acids giving rise to these intermediates are termed as glucogenic. Those amino acids degraded to yield acetyl CoA or acetoacetate are termed ketogenic since these compounds are used to synthesize ketone bodies.

Some amino acids are both glucogenic and ketogenic (For example, phenylalanine, tyrosine, tryptophan and threonine. The product ammonia is excreted acid is always the removal of its after conversion to urea or other products and the carbon skeleton is degraded to CO_2 releasing energy.

Deamination

The citric acid cycle is not only a pathway for oxidation of two-carbon units—it is also a major pathway for interconversion of metabolites arising from transamination and deamination of amino acids. It also provides the substrates for amino acid synthesis by transamination, as well as for gluconeogenesis and fatty acid synthesis. Because it functions in both oxidative and synthetic processes, it is amphibolic. Deamination is the process by which amino acids are broken down if there is an excess of protein intake. Deamination is the removal of an amine group from a molecule. Enzymes that catalyse this reaction are called deaminases.

In the human body, deamination takes place primarily in the liver, however glutamate is also deaminated in the kidneys. The amino group is removed from the amino acid and converted to ammonia. Ammonia is toxic to the human system, and enzymes convert it to urea or uric acid by addition of carbon dioxide molecules in the urea cycle, which also takes place in the liver. Urea and uric acid can safely diffuse into the blood and then be excreted in urine.

Oxidative Deamination

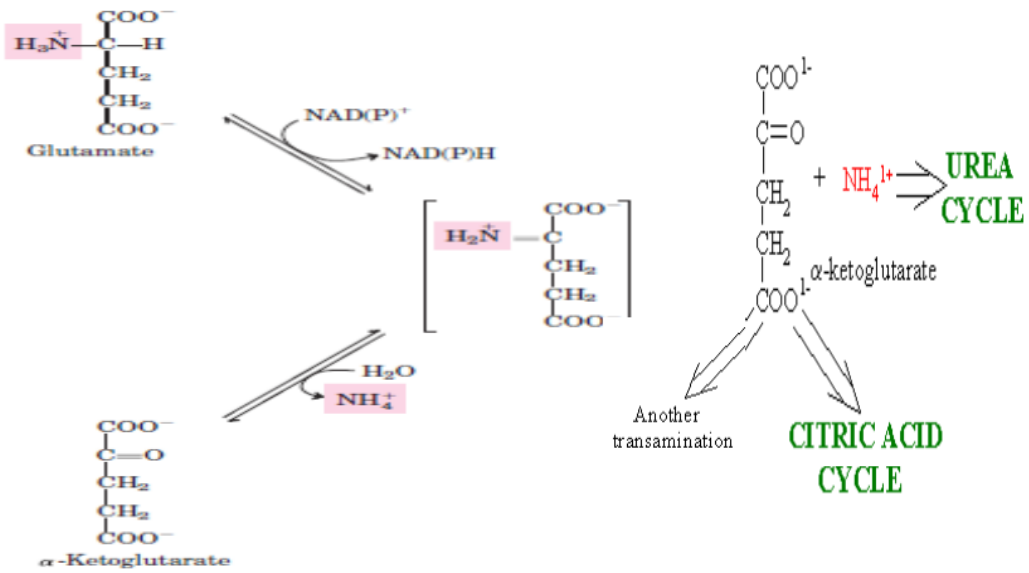
Oxidative deamination occurs primarily on glutamic acid because glutamic acid is the end product of many transamination reactions. During oxidative deamination, an amino acid is converted into the corresponding keto acid by the removal of the amine functional group as

ammonia and the amine functional group is replaced by the ketone group. The ammonia eventually goes into the urea cycle.

The glutamate dehydrogenase is allosterically controlled by ATP and ADP. ATP acts as an inhibitor whereas ADP is an activator.

During transamination α-amino groups of most amino acids are transferred to α- ketoglutarate forming L-glutamate.

- These amino groups are next removed from glutamate as ammonia to prepare them for excretion.
- In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes oxidative deamination catalyzed by L-glutamate dehydrogenase.
- It is one of few enzymes that can use either NAD or NADP as cofactor.
- L-glutamate dehydrogenase is a very active enzyme, present in most mammalian tissues in the mitochondrial matrix.
- The combined action of an aminotransferase and glutamate dehydrogenase is referred to as transdeamination.
- A few amino acids bypass the transdeamination pathway and undergo direct oxidative deamination. Example: L-amino acid oxidases of liver and kidney produces NH3 and α-keto acid directly, using FMN as a cofactor (through α-imino acid) FMNH2 is converted to FMN, using O2 and produces H2O2 which is decomposed by catalase.
- The -ketoglutarate formed from glutamate deamination can be used in the citric acid cycle for glucose synthesis or for another transamination.



Reaction catalyzed by glutamate dehydrogenase

• Glutamate Dehydrogenase catalyzes a major reaction that effects net removal of N from the amino acid pool which is one of the few enzymes that can use NAD + or NADP + as co-factor.

• Oxidation at the α-carbon is followed by hydrolysis, releasing NH_4 +.

This enzyme is allosterically inhibited by GTP and ATP .Glutamate Dehydrogenase Reaction – Direction of reactions: The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia, and the ratio of oxidized to reduced coenzymes.

- For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia
- Allosteric regulators: guanosine triphosphate and ATP are allosteric inhibitors of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator.
- Thus, when energy levels are low in the cell, amino acid degradation by glutamate dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

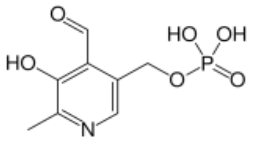
Non-oxidative Deamination

- Occurs in liver and kidney.

- The amino groups of hydroxy amino acids as serine, therionine and hydroxy proline are enzymatically removed.
- This has low value in deamination mechanisms.
- Amino acids such as serine and histidine are deaminated non-oxidatively the other reactions involved in the catabolism of amino acids are decarboxylation, transulfuration, desulfuration, dehydration etc.

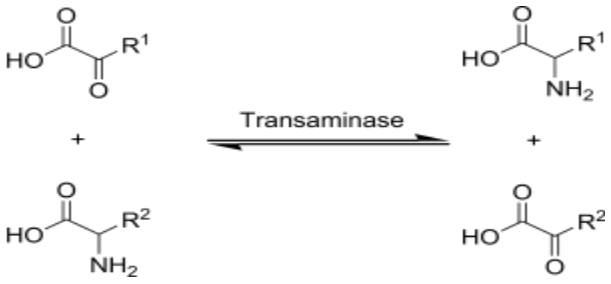
Transamination

Transamination (or aminotransfer) is a chemical reaction between two molecules. One is an amino acid, which contains an amine (NH_2) group. The other is a keto acid, which contains a keto (=O) group. In transamination, the NH_2 group on one molecule is exchanged with the =O group on the other molecule. The amino acid becomes a keto acid, and the keto acid becomes an amino acid.



Structure of Pyridoxal Phospahte

The products usually are either, alanine, aspartate or glutamate, since their corresponding alpha-keto acids are produced through metabolism of fuels. Serine and threonine are the only two amino acids that do not always undergo transamination and rather use serine or threonine dehydrogenase.

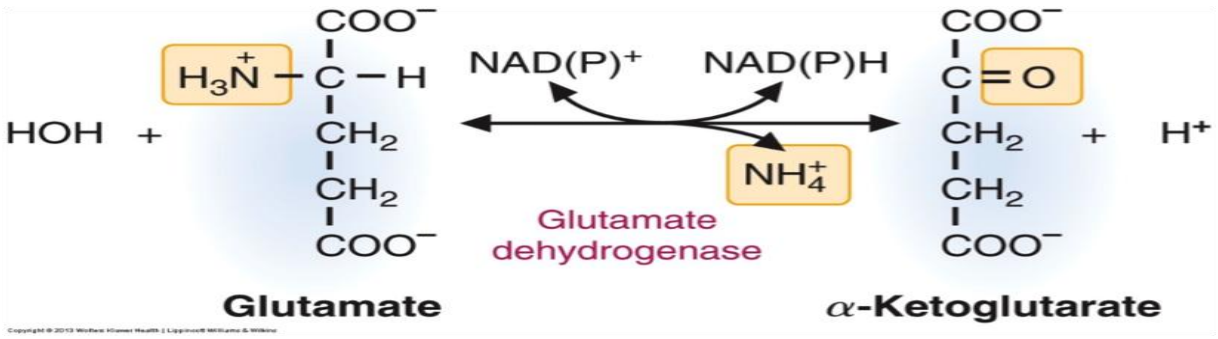


Reactions catalyzed by transaminase

Formation and Transport of Ammonia

The amino groups of most amino acids are ultimately transferred to α-ketoglutarate by transamination. Release of this nitrogen as ammonia is catalyzed by L-glutamate dehydrogenase, which is widely distributed in mammalian tissues. Figure 5 explains the conversion of glutamate

to α- Ketoglutarare. Glutamate dehydrogenase uses NAD^+ or $NADP^+$ as cosubstrate. The reaction is reversible.



Reactions catalyzed by Glutamate dehydrogenase

Intestinal bacteria produce ammonia from dietary protein as well as from the urea present in fluids secreted into gastrointestional tract. This ammonia is absorbed from the intestine into portal vein blood. Under normal conditions the liver promptly removes the ammonia from the portal blood.

Ammonia is produced in the kidney from intracellular amino acid, glutamine catalyzed by renal glutaminase. Ammonia production by the kidney is highly increased in metabolic acidosis and depressed in alkalosis.

Ammonia is present only in traces in blood (10-20µg /100ml) because it is rapidly removed from the circulation by the liver and converted to glutamine or urea.

Disposal of ammonia- urea cycle

Urea produced in the liver freely diffuses and is transported in blood to kidneys, and excreted. A small amount of urea enters the intestine where it is broken down to CO_2 and NH_3 by the bacterial enzyme urease. This ammonia is either lost in the feces or absorbed into the blood. In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NHs. Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Urea cycle:

Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia (as described above), is toxic to the body. It is converted to

urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine. Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Hensele it (1932), hence it is known as Krebs-Hensele it cycle. The individual reaction show ever was described in more detail later on by Ratner and Cohen. Urea has two amino (-NH) groups, one derived from NH and the other from aspartate. Carbon atom is supplied by CO2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol. The details of urea cycle are described

1. Synthesis of carbamoyl phosphate:

Carbamoyl phosphate synthase I (CPS l) of mitochondria catalyses the condensation of NH ions with CO2 to form carbamoyl phosphate. This step consumes two ATP and is irreversible, and rate-limiting. CPS I requires N-acetyl glutamate for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II) - involved in pyrimidine synthesis-is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. Formation of citrulline:

Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerateda nd used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (Thev are never found in protein structure due to lack of codons).Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. Synthesis of arginosuccinate:

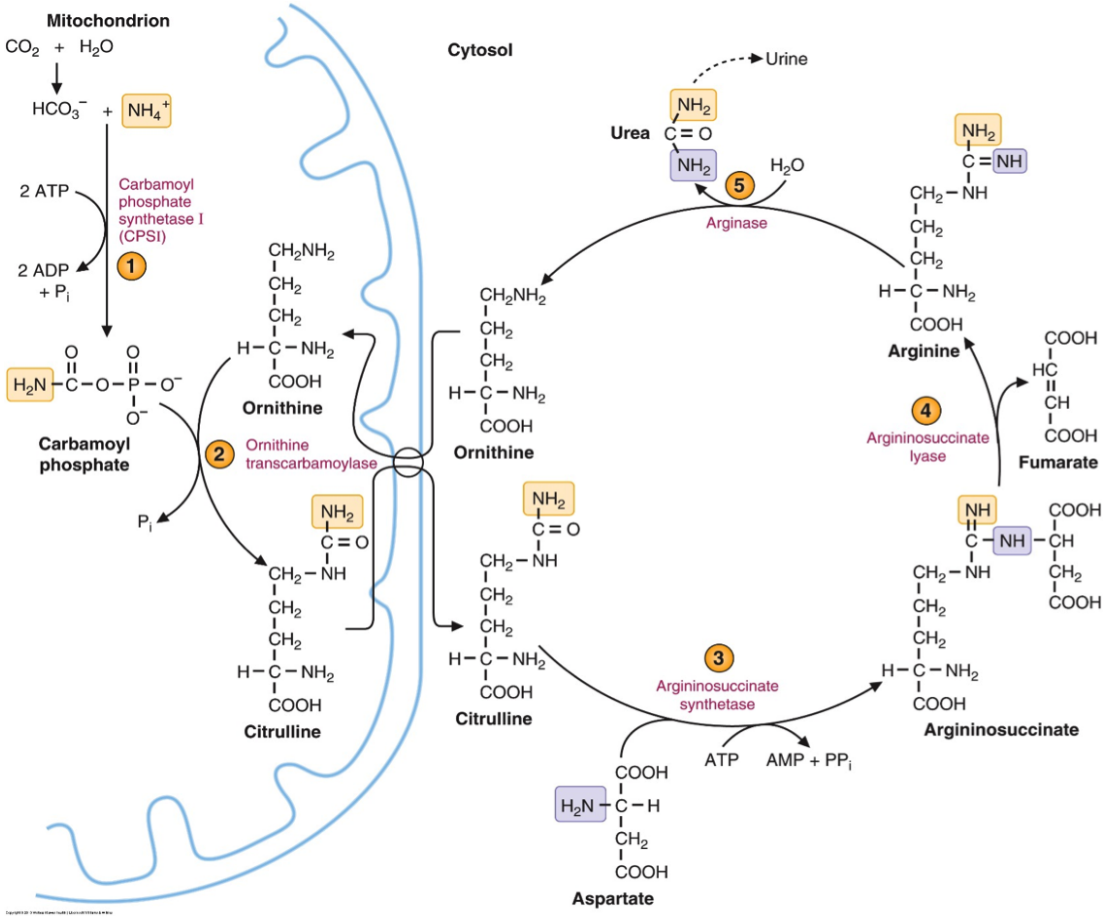
Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

4. Cleavage of arginosuccinate:

Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesies etc.

5. Formation of urea:

Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co_2 and Mn_2+. Ornithine and lysine compete with arginine (competitive inhibition). Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.



The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce argino succinate which equals to 2 ATP. Hence 4 ATP are actually consumed.

NH_4 + CO_2 + Aspartate + 3ATP -----+ Urea + Fumarate+ 2 ADP + 2 Pi + AMP + PPi

Regulation of urea cycle

The first reaction catalysed by carbamoyl phosphate synthase I (CPS l) is rate limiting reaction or committed step in urea synthesis. CPS I is allosterically activated by N-acetylglutamate (NAC). It is synthesized from glutamate and acetyl CoA by synthase and degraded by a hydrolase. The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAC. The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis. Carbamoyl phosphate synthase I and glutamate dehydrogenase are localized in the mitochondria. They coordinate with each other in the formation of NH_1, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

DECARBOXYLATION

The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines. The enzymes, amino acid decarboxylases are pyridoxal phosphate dependent enzymes. Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilize carbon α-carbanion formed by the cleavage of bond between carboxyl andα. The physiologically active amines epinephrine, nor-epinephrine, dopamine atom, amino buyrate and histamine are formed through decarboxylation of γ serotonin, the corresponding precursor amino acids.

AMINO ACID CATABOLISM

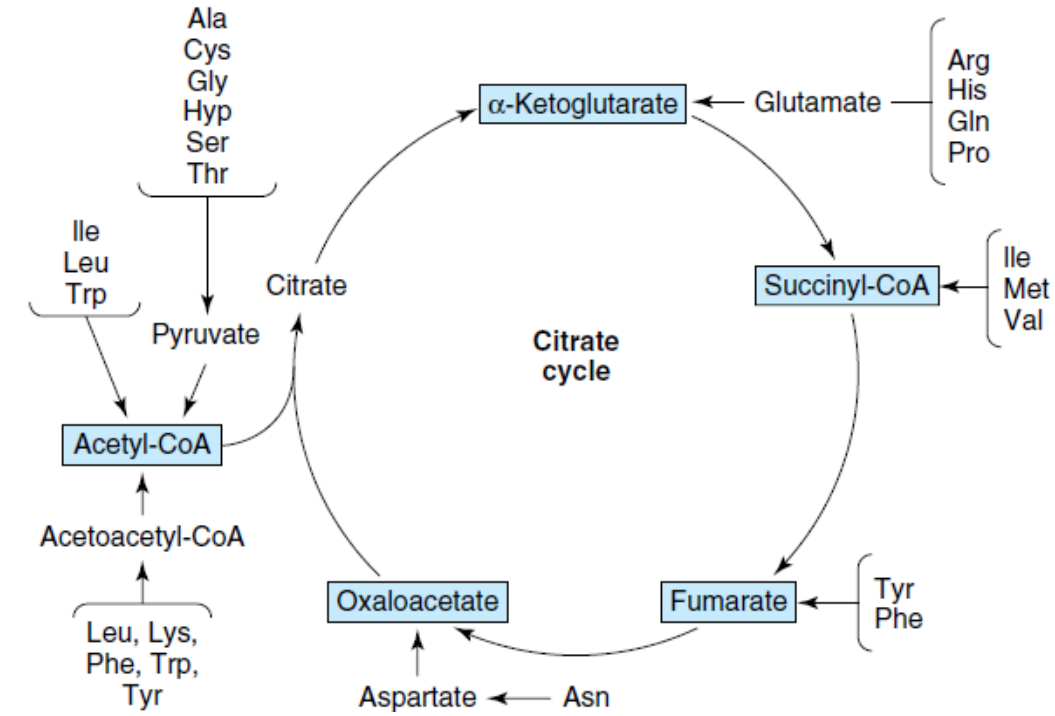
Removal of α-amino nitrogen by transamination is the first catabolic reaction of amino acids except in the case of proline, hydroxyproline, threonine, and lysine. The residual hydrocarbon skeleton is then degraded to amphibolic intermediates.

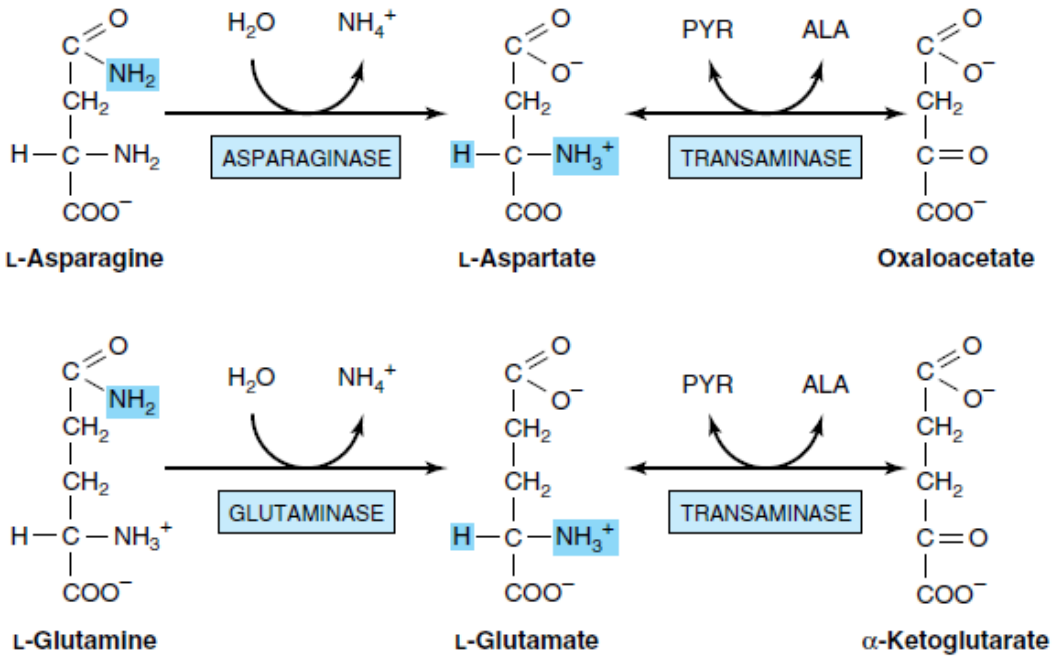
Asparagine, Aspartate, Glutamine and Glutamate

All four carbons of asparagine and aspartate form oxaloacetate. Analogous reactions convert glutamine and glutamate to α -ketoglutarate. Since the enzymes also fulfill anabolic functions, no metabolic defects are associated with the catabolism of these four amino acids.

Proline

Proline forms dehydroproline, glutamate-γ-semialdehyde, glutamate, and, ultimately, ketoglutarate. The metabolic block in type I hyperprolinemia is at proline dehydrogenase.


Ala
Cys
Gly
Hyp
Ser
Thr
Ile
Leu
Trp
Pyruvate
Citrate
α-Ketoglutarate
Glutamate
Arg
His
Gln
Pro
Succinyl-CoA
Ile
Met
Val
Citrate cycle
Acetyl-CoA
Acetoacetyl-CoA
Leu, Lys, Phe, Trp, Tyr
Oxaloacetate
Fumarate
Tyr
Phe
Aspartate
Asn


H2O
NH4+
ASPARAGINASE
PYR
ALA
TRANSAMINASE
L-Asparagine
L-Aspartate
Oxaloacetate
GLUTAMINASE
L-Glutamine
L-Glutamate
α-Ketoglutarate

There is no associated impairment of hydroxyproline catabolism. The metabolic block in **type II hyperprolinemia** is at **glutamate-semialdehyde dehydrogenase,** which also functions in hydroxyproline catabolism. Both proline and hydroxyproline catabolism thus are affected and 1-pyrroline-3-hydroxy-5-carboxylate is excreted.

Arginine and Ornithine.

Arginine is converted to ornithine, glutamate -semialdehyde, and then α-ketoglutarate. Mutations in ornithine aminotransferase elevate plasma and urinary ornithine and cause gyrate atrophy of the retina. Treatment involves restricting dietary arginine. In hyperornithinemia-hyperammonemia syndrome, a defective mitochondrial ornithine-citrulline antiporter impairs transport of ornithine into mitochondria for use in urea synthesis.

Histidine

Catabolism of histidine proceeds via urocanate, 4-imidazolone-5-propionate, and *N*-for imiminoglutamate. For mimino group transfer to tetrahydrofolate forms glutamate, then α-ketoglutarate. In folic acid deficiency, group transfer is impaired and Figlu is excreted. Excretion of Figlu following a dose of histidine thus has been used to detect folic acid deficiency. Benign disorders of histidine catabolism include histidinemia and urocanic aciduria associated with impaired histidase.

Glycine.

Glycine undergoes oxidative deamination by glycines ynthaseto liberate NH_4^+,CO_2 and one carbon fragments N^5, N^{10}-methylenTHF.This provides a major route for glycine breakdown in mammals. Glycine synthase is a multienzyme complex and requires PLP, NAD+ and THF for its activity. This reaction is reversible and, therefore, glycine can be generated from onecarbon unit (methylene fragment of THF). Glycine is reversibly converted to serine by THF dependent serine hydroxyl methyl transferase. Pyruvate produced from serine by serine dehydratase, serves as a precursor for glucose. Serine is degraded to glyoxylate which undergoes transamination to give back glycine. Glyoxylate is also converted to oxalate, an excretory product and formate which enters one carbon pool.

Serine

Following conversion to glycine, catalyzed by serine hydroxyl methyl transferase, serine catabolism merges with that of glycine. Epidemiologic data suggesting a relationship between

plasma homocysteine and cardiovascular disease, whether homocysteine represents a causal cardiovascular risk factor remains controversial.

Threonine

Threonine is cleaved to acetaldehyde and glycine. Oxidation of acetaldehyde to acetate is followed by formation of acetyl-CoA.

4-Hydroxyproline

Catabolism of 4-hydroxy-L-proline forms, successively, L-Δ1-pyrroline-3-hydroxy-5-carboxylate, γ-hydroxy-L-glutamate-γ-semialdehyde, erythro-γ-hydroxy-L-glutamate, and α-keto-γ-hydroxyglutarate. An aldol-type cleavage then forms glyoxylate plus pyruvate. A defect in 4-hydroxyproline dehydrogenase results in hyperhydroxyprolinemia, which is benign. There is no associated impairment of proline catabolism.

Lysine

Lysine first forms a Schiff base with α-ketoglutarate, which is reduced to **saccharopine.** In one form of **periodic hyperlysinemia**, elevated lysine competitively inhibits liver **arginase**, causing hyperammonemia. Restricting dietary lysine relieves the ammonemia, whereas ingestion of a lysine load precipitates severe crises and coma. In a different **periodic hyperlysinemia,** lysine catabolites accumulate, but even a lysine load does not trigger hyperammonemia. In addition to impaired synthesis of saccharopine, some patients cannot cleave saccharopine.

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DEPARTMENT OF BIOCHEMISTRY
III B.Sc BIOCHEMISTRY
BATCH: 2015 - 2018

SUBJECT: METABOLIC PATHWAYS **SUBJECT CODE: 15BCU501** **UNIT: IV**

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

S. No	Question	Option A	Option B	Option C	Option D	Answer
	The transaminase activity needs the coenzyme	ATP	B_6-PO_4	FAD^+	NAD^+	B_6-PO_4
	With regard to proteolytic enzymes, the following statement is correct	Trypsin acts as an activator for all zymogens	Trypsin is an exopeptidase and liberates free amino acids	Pepsin is secreted by the pancreas	Chymotrypsin acts in the stomach on peptide bonds formed by glycine	Trypsin acts as an activator for all zymogens
	The enzyme which converts phenyl alanine into tyrosine is	Phenylalanine decarboxylase	Phenylalanine hydroxylase	Transaminase	Decarboxylase	Phenylalanine hydroxylase
	The reaction which completely removes amino group from amino acid is	Transamination	Oxidative deamination	Non- oxidative deamination	Decorboxylation	Oxidative deamination
	The ketogenic amino acid is	Alanine	leucine	glycine	arginine	leucine
	The transfer of amino group from amino acid to α keto acid is	Transamination	decarboxylation	dehydrogenation	Deamination	transamination
	The enzyme involved in the hydrolyses of arginine to urea and ornithin is	Ornithin trans carbamoylase	Arginase	argino succinate synthase	trans carbamoylase	Arginase
	The amphibolic intermediate formed from glycine is	citrate	fumarate	pyruvate	succinate	pyruvate
	The coenzyme for L- amino acid oxidases is	FMN	pyridoxal phosphate	NAD	TPP	FMN
	Which of the following types of the amino acids are involved in the synthesis of the nucleic acid?	non essential amino acids	basic amino acids	aromatic amino acids	aliphatic hydrophobic amino acids	non essential amino acids
	The two nitrogen atoms in urea arise from	ammonia and aspartic acid	ammonia and glutamine	glutamine and alanine	glutamine and aspartic acid	ammonia and aspartic acid
	The coenzyme for non oxidative deaminase is	TPP	NAD^+	FAD	PLP	PLP
	Ammonia is detoxified in brain chiefly as	Urea	Uric acid	Creatinine	Glutamine	Glutamine
	The side chain of tryptophan is cleaved to yield	Serine	Glycine	alanine	cysteine	alanine
	An amino acid not involved in urea cycle is	arginine	histidine	ornithine	citrulline	histidine
	Formation of ammonia solely occurs in	the kidney	the spleen	the liver	The pancreas	the kidney
	The coenzyme required for amino acid decarboxylation is	FMN	FAD	NAD	pyridoxal phosphate	pyridoxal phosphate
	The metabolism of L-tryptophan results in	uric acid	creatinine	a vitamin	allantoin	a vitamin
	Glutamine is the chief detoxifying product of ammonia in	liver	brain	kidney	intestine	brain
	Uric acid is the catabolic end product of	porphyrins	purines	pepinidines	pyridoxine	purines
	Protein nitrogen is fixed in urea by way of	transamination	transamidation	oxidative deamination	hydrolysis	transamination
	Argininosuccinicaciduria is due to the absence of	arginine	argininosuccinase	ornithine transcarbamoylase	carbamoyl phosphate synthetase	argininosuccinase
	Oxidative deamination of glutamate	requires NAD^+	glutamate dehydrogenase	requires FMN	forms α-ketoglutarate	glutamate dehydrogenase
	During convalescing period the body is under	negative nitrogen balance	positive nitrogen balance	acidic nitrogen balance	basic nitrogen balance	positive nitrogen balance
	Non oxidative deamination of amino acids yields	keto acids	amino acids	aliphatic acids	aliphatic alcohol	keto acids
	Transamination of oxaloacetate yields	alanine	glutamic acid	serine	valine	glutamic acid
	Uric acid is not an end product in	man	rat	birds	amphibians	man
	Which of the following statements is not true about transamination?	pyridoxal phosphate acts as a coenzyme	alpha keto acid acts as a substrate	controlled by allosteric effectors	ammonia is neither consumed nor produced	ammonia is neither consumed nor produced
	During old age there is	positive N_2 balance	negative N_2 balance	N_2 balance	dynamic equilibrium	negative N_2 balance
	In human nitrogen of amino acid is removed as	ammonia	uric acid	urea	glutamine	urea
	Formation of melanin from tyrosine requires the action of	dopa carboxylase	peroxidase	diamine oxidase	tyrosinase	tyrosinase
	The important methyl group donor in metabolic reaction is	methionine	cysteine	S-adenosyl methionine	tyrosine	S-adenosyl methionine
	The inborn errors of metabolism caused by the deficiency of tyrosinase is	albinism	alcaptonurea	tyrosinosis	phenyl ketonurea	albinism
	The enzyme involved in conversion of ammonia and glutamate to glutamine is	Glutaminase	Glutamine synthetase	Glutamate Dehydrogenase	Glutamate oxidase	Glutamine synthetase
	An amino acid which is both glycogenic and ketogenic is	alanine	arginine	leucine	lysine	lysine
	Uric acid is	highly soluble in water	hydrolysed by water	sparingly soluble in water	insoluble in water	insoluble in water
	Reductive amination of α- ketoglutarate is catalysed by	glutamine synthetase	aminotransferase	glutamate dehydrogenase	hydroxyl methyl transferase	glutamate dehydrogenase
	Oxidative deamination is catalysed by	L- aminoacid oxidase	argininosuccinase	transcarbamoylase	glutamate dehydrogenase	L- aminoacid oxidase
	Which is not nutritionally essential but it is formed from methionine which is nutritionally essential	glycine	proline	serine	cysteine	cysteine
	Which enzyme promote the adenylation and deadenylation reaction	uridylyl transferase	adenylase	adenylyl transferase	uridine	adenylyl transferase
	Which is the cyclised derivative of glutamate	histidine	proline	adenine	guanine	proline
	Major precursor for biosynthesis of porphyrins	glycine	glutamate	glutamine	aspartate	glycine
	End product of tyrosine metabolism is	acetyl coA	pyruvate	catecholamines	succinate	catecholamines
	Decarboxylation of glutamate give rise to	histamine	serotonin	cimetidine	GABA	GABA
	The pyrimidine ring is synthesized as	inosinate	orotate	PRPP	uridine	orotate
	Which aminoacid is important precursor for purine synthesis	glycine	glutamate	aspartate	lysine	glycine
	Which aminoacid is important precursor for pyrimidine synthesis	glycine	glutamate	aspartate	lysine	aspartate
	The enzyme involved in the synthesis of 5 phospho ribosyl amine from PRPP	PRPP synthetase	glutamine PRPP amydo transferase	adelylosuccinate dehydrogenase	trans carbamoylase	glutamine PRPP amydo transferase
	Krebs-Hensleit cycle is	TCA cycle	electron transport chain	urea cycle	creatinine cycle	urea cycle
	Phenylalanine is transaminated to	pyruvate	phenylpyruvate	acetyl CoA	tyrosine	phenylpyruvate
	Metabolic disorder of tryptophan catabolism is	Hartnup disease	Gaucher's disease	alkaptonuria	phenylketonuria	Hartnup disease
	The unwanted amino acids abstracted from the tissues are either used up by the tissue or in the liver converted into	Ammonia	Urea	Ammonium salts	Uric acid	Urea
	Amino acids provide the nitrogen for the synthesis of	The bases of the phospholipids	Uric acid	Glycolipids	Chondroitin sulphates	The bases of the phospholipids
	Synthesis of glutamine is accompanied by the hydrolysis of	ATP	ADP	TPP	Creatin phosphate	ATP
	Sulphur containing amino acids after catabolism produces a substance which is excreted:	SO_2	HNO_3	H_2SO_4	H_3PO_4	H_2SO_4
	Most amino acids are substrates for transamination except	Alanine	Threonine	Serine	Valine	Threonine
	Oxidative conversion of many amino acids to their corresponding -ketoacids occurs in mammalian:	Liver and kidney	Adipose tissue	Pancreas	Intestine	Liver and kidney
	The α-ketoacid is decarboxylated by H2O2 forming a carboxylic acid with one carbon atom less in the absence of the enzyme:	Catalase	Decarboxylase	Deaminase	Phosphatase	Catalase
	From dietary protein as well as from the urea present in fluids secreted into the gastrointestinal tract intestinal bacteria produce	Carbondioxide	Ammonia	Ammonium sulphate	Creatine	Ammonia
	The conversion of inosinate to adenylate requires the insertion of an amino group derived from ----------	aspartate	glutamate	arginine	glutamine	aspartate

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DEPARTMENT OF BIOCHEMISTRY

SUBJECT : METABOLIC PATHWAYS
SEMESTER : V
SUBJECT CODE : 15BCU501 CLASS : III B.Sc.BC

UNIT V-- COURSE MATERIAL

Metabolism of purine and pyrimidine nucleotides

Introduction, biomedical importance, biosynthesis of purine and pyrimidine nucleotides, de novo synthesis of purines and pyrimidines, salvage pathways , catabolism of purines and pyrimidines.

Inter relationship of carbohydrate, protein and fat metabolism. TCA cycle as a central core in the inter relationships in metabolism and inter conversion of major food stuffs - Carbohydrate, fats and proteins.

TEXT BOOKS

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Robert K. Murray, Daryl K. Granner Peter A. Mayes Victor W. Rodwell, 2012, Harper's Biochemistry, 29th Ed, Mc Graw-Hill Medical, London.

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UNIT-V: METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES

Introduction

The metabolic requirements for the nucleotides and their cognate bases can be met by both dietary intake and synthesis de novo from low molecular weight precursors. Indeed, the ability to salvage nucleotides from sources within the body alleviates any nutritional requirement for nucleotides, thus the purine and pyrimidine bases are not required in the diet. The salvage pathways are a major source of nucleotides for synthesis of DNA, RNA and enzyme co-factors.

The biosynthesis of purines and pyrimidines is stringently regulated and coordinated by feedback mechanisms that ensure their production in quantities and at times appropriate to varying physiologic demand. Genetic diseases of purine metabolism include gout, Lesch-Nyhan syndrome, adenosine deaminase deficiency, and purine nucleoside phosphorylase deficiency. By contrast, apart from the orotic acidurias, there are few clinically significant disorders of pyrimidine catabolism.

Nucleic acid biosynthesis

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

The de novo pathways for purine and pyrimidine biosynthesis appear to be nearly identical in all living organisms. Notably, the free bases guanine, adenine, thymine, cytidine, and uracil are not intermediates in these pathways; that is, the bases are not synthesized and then attached to ribose, as might be expected. The purine ring structure is built up with one or a few atoms at a time, attached to ribose throughout the process. The pyrimidine ring is synthesized as orotate, attached to ribose phosphate, and then converted to the common pyrimidine nucleotides required in nucleic acid synthesis. Although the free bases are not intermediates in the de novo pathways, they are intermediates in some of the salvage pathways.

Purines & Pyrimidines are dietarily nonessential

Human tissues can synthesize purines and pyrimidines from amphibolic intermediates. Ingested nucleic acids and nucleotides, which therefore are dietarily nonessential, are degraded in the intestinal tract to mononucleotides, which may be absorbed or converted to purine and pyrimidine bases. The purine bases are then oxidized to uric acid, which may be absorbed and excreted in the urine. While little or no dietary purine or pyrimidine is incorporated into tissue nucleic acids, injected compounds are incorporated. The incorporation of injected [^{3}H] thymidine into newly synthesized DNA thus is used to measure the rate of DNA synthesis.

Biosynthesis of purine

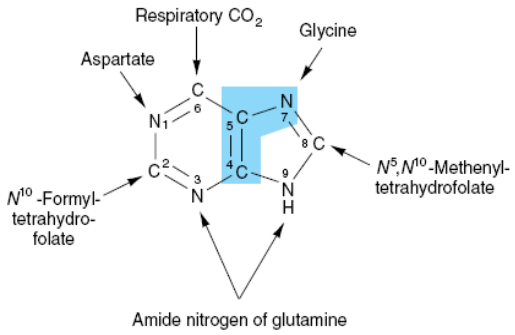
Purine and pyrimidine nucleotides are synthesized *in vivo* at rates consistent with physiologic need. Intracellular mechanisms sense and regulate the pool sizes of nucleotide triphosphates (NTPs), which rise during growth or tissue regeneration when cells are rapidly dividing. Early investigations of nucleotide biosynthesis employed birds, and later ones used *Escherichia coli*. Isotopic precursors fed to pigeons established the source of each atom of a purine base and initiated study of the intermediates of purine biosynthesis.

Antifolate Drugs or Glutamine Analogs Block Purine Nucleotide Biosynthesis

The carbons added in reactions are contributed by derivatives of tetrahydrofolate. Purine deficiency states, which are rare in humans, generally reflect a deficiency of folic acid. Compounds that inhibit formation of tetrahydrofolates and therefore block purine synthesis have been used in cancer chemotherapy. Inhibitory compounds and the reactions they inhibit include azaserine, diazanorleucine, 6-mercaptopurine, and mycophenolic acid.

Many compounds contribute to the purine ring of the nucleotides.

- N_1- of purine is derived from amino group of aspartate.
- C_2- and Cs arise from formate of N10-formyl THF.
- N_3- and N9 are obtained from amide group of glutamine
- C_4, C_5 and N_7 are contributed by glycine.
- C_6 directly comes from CO_2.



Sources of the nitrogen and carbon atoms of the purine ring

Sources of the nitrogen and carbon atoms of the purine ring, It should be remembered that purine bases are not synthesized as such, but they are formed as ribonucleotides. The purines are built upon a pre-existing ribose 5-phosphate. Liver is the major site for purine nucleotide synthesis. Erythrocytes, polymorphonuclear leukocytes and brain cannot produce purines. Three processes contribute to purine nucleotide biosynthesis. These are, in order of decreasing importance: (1) synthesis from amphibolic intermediates (synthesis de novo), (2) phosphoribosylation of purines, and (3) phosphorylation of purine nucleosides.

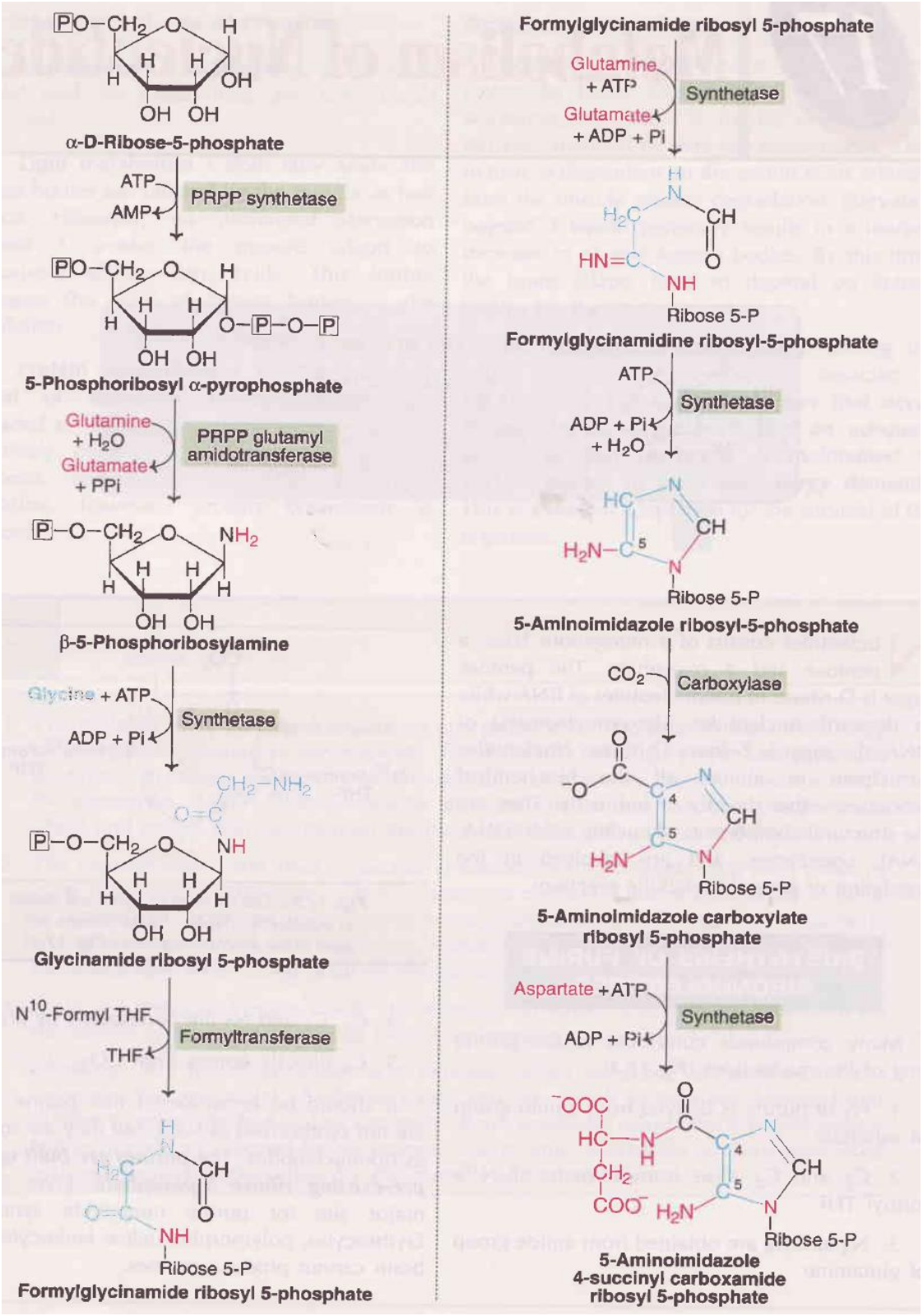
AMP & GMP Feedback-Regulate PRPP Glutamyl Amidotransferase

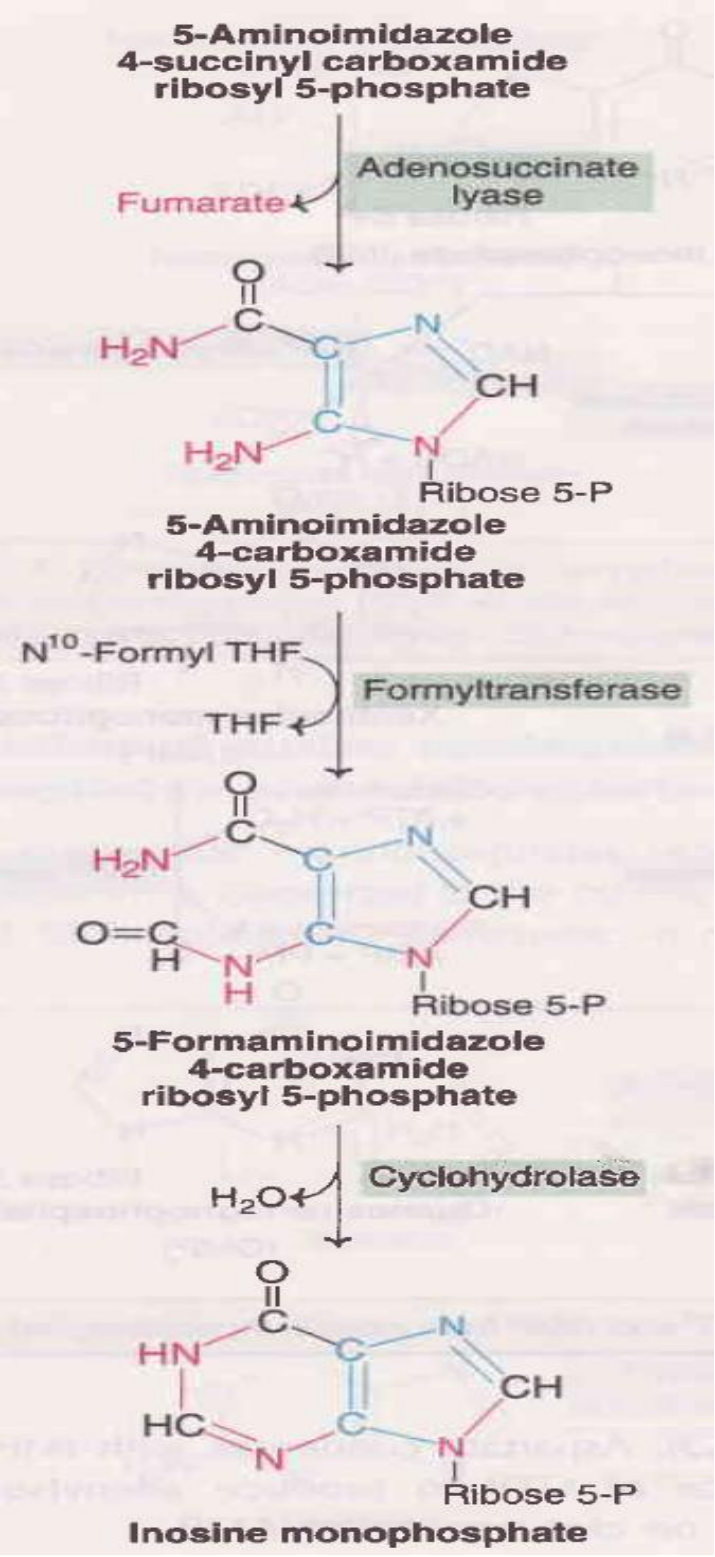
Since biosynthesis of IMP consumes glycine, glutamine, tetrahydrofolate derivatives, aspartate, and ATP,it is advantageous to regulate purine biosynthesis. The major determinant of the rate of de novo purine nucleotide biosynthesis is the concentration of PRPP, whose pool size depends on its rates of synthesis, utilization, and degradation. The rate of PRPP synthesis depends on the availability of ribose 5-phosphate and on the activity of PRPP synthase, an enzyme sensitive to feedback inhibition by AMP, ADP, GMP, and GDP.

AMP & GMP Feedback-Regulate Their Formation from IMP

The metabolic pathway for the synthesis of inosine monophosphate, the parent purine nucleotide (PRPP-Phosphoribosyl pytophosphate; PPi-Pyrophosphate), two mechanisms regulate conversion of IMP to GMP and AMP. AMP and GMP feedback-inhibit adenylosuccinate synthase and IMP dehydrogenase, respectively. Furthermore, conversion of IMP to adenylosuccinate en route to AMP requires GTP, and conversion of xanthinylate (XMP) to GMP requires ATP. This cross-regulation between the pathways of IMP metabolism thus serves to decrease synthesis of one purine nucleotide when there is a deficiency of the other nucleotide.

AMP and GMP also inhibit hypoxanthine-guanine phosphoribosyl transferase, which converts hypoxanthine and guanine to IMP and GMP, and GMP feedback-inhibits PRPP glutamyl amidotransferase


α-D-Ribose-5-phosphate
ATP
AMP
PRPP synthetase
5-Phosphoribosyl α-pyrophosphate
Glutamine + H2O
Glutamate + PPi
PRPP glutamyl amidotransferase
β-5-Phosphoribosylamine
Glycine + ATP
ADP + Pi
Synthetase
Glycinamide ribosyl 5-phosphate
N10-Formyl THF
THF
Formyltransferase
Ribose 5-P
Formylglycinamide ribosyl 5-phosphate
Formylglycinamide ribosyl 5-phosphate
Glutamine + ATP
Glutamate + ADP + Pi
Synthetase
Ribose 5-P
Formylglycinamidine ribosyl-5-phosphate
ATP
ADP + Pi + H2O
Synthetase
Ribose 5-P
5-Aminoimidazole ribosyl-5-phosphate
CO2
Carboxylase
Ribose 5-P
5-Aminoimidazole carboxylate ribosyl 5-phosphate
Aspartate + ATP
ADP + Pi
Synthetase
Ribose 5-P
5-Aminoimidazole 4-succinyl carboxamide ribosyl 5-phosphate



The metabolic pathway for the synthesis of inosine monophosphate, the parent purine nucleotide (PRPP-Phosphoribosyl pytophosphate ; PPi-Pyrophosphate)

Reactions

- Ribose 5-phosphate, produced in the hexose monophosphate shunt of carbohydrate metabolism is the starting material for purine nucleotide synthesis. It reacts with ATP to form phosphoribosyl pyrophosphate (PRPP).
- Glutamine transfers its amide nitrogen to PRPP to replace pyrophosphate and produce 5-phosphoribosylamine. The enzyme PRPP glutamyl amidotransferase is controlled by feedback inhibition of nucleotides (IMP, AMP and GMP). This reaction is the 'committed step' in purine nucleotide biosynthesis.
- Phosphoribosylamine reacts with glycine in the presence of ATP to form glycinamide ribosyl 5-phosphate or glycinamide ribotide (GAR).
- N^{10}-Formyl tetrahydrofolate donates the formyl group and the product formed is formylglycinamide ribosyl 5-phosphate.
- Glutamine transfers the second amido amino group to produce formyl-glycinamidine ribosyl 5-phosphate.
- The imidazole ring of the purine is closed in an ATP dependent reaction to yield 5-aminoimidazole ribosyl S-phosphate.
- Incorporation of CO_2 (carboxylation) occurs to yield amino imidazole carboxylate ribosyl 5-phosphate. This reaction does not require the vitamin biotin and/or ATP which is the case with most of the carboxylation reactions.
- Aspartate condenses with the product in reaction 7 to form aminoimidazole 4-succinyl carboxamide ribosyl S-phosphate.
- Adenosuccinate lyase cleaves off fumarate and only the amino group of aspartate is retained to yield aminoimidazole4 –carboxamide ribosyl 5-phosphate.
- N^{1O}-Formyl tetrahydrofolate donates a one-carbon moiety to produce formaminoimidazole 4-carboxamide ribosyl 5-phosphate. With this reaction, all the carbon and nitrogen atoms of purine ring are contributed by the respective sources.
- The final reaction catalysed by cyclohydrolase leads to ring closure with elimination of water molecule. The product obtained is inosine monophosphate(lMP), the parent purine nucleotide from which other purine nucleotides can be synthesized.

Reduction of Ribonucleoside Diphosphates forms Deoxyribonucleoside Diphosphates

Reduction of the 2′-hydroxyl of purine and pyrimidine ribonucleotides, catalyzed by the ribonucleotide reductase complex, forms deoxyribonucleoside diphosphates (dNDPs). The enzyme complex is active only when cells are actively synthesizing DNA. Reduction requires thioredoxin, thioredoxin reductase, and NADPH. The immediate reductant, reduced thioredoxin, is produced by NADPH: thioredoxin reductase. Reduction of ribonucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs) is subject to complex regulatory controls that achieve balanced production of deoxyribonucleotides for synthesis of DNA

As in the tryptophan and histidine biosynthetic pathways, the enzymes of IMP synthesis appear to be organized as large, multienzyme complexes in the cell. Once again, evidence comes from the existence of single polypeptides with several functions, some catalyzing nonsequential steps in the pathway. In eukaryotic cells ranging from yeast to fruit flies to chickens, are catalyzed by a multifunctional protein. An additional multifunctional protein catalyzes steps 10 and 11.

In bacteria, these activities are found on separate proteins, but a large noncovalent complex may exist in these cells. The channeling of reaction intermediates from one enzyme to the next permitted by these complexes is probably especially important for unstable intermediates such as 5-phosphoribosylamine. Conversion of inosinate to adenylate requires the insertion of an amino group derived from aspartate, this takes place in two reactions similar to those used to introduce N-1 of the purine ring. A crucial difference is that GTP rather than ATP is the source of the high-energy phosphate in synthesizing adenylosuccinate. Guanylate is formed by the NAD-requiring oxidation of inosinate at C-2, followed by addition of an amino group derived from glutamine. ATP is cleaved to AMP and PPi in the final step.

Salvage pathway

The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources. The purines can be directly converted to the corresponding nucleotides, and this process is known as 'salvage pathway'. Conversion of purines, their ribonucleosides, and their deoxyribonucleosides to mononucleotides involves so called “salvage reactions” that require far less energy than de novo

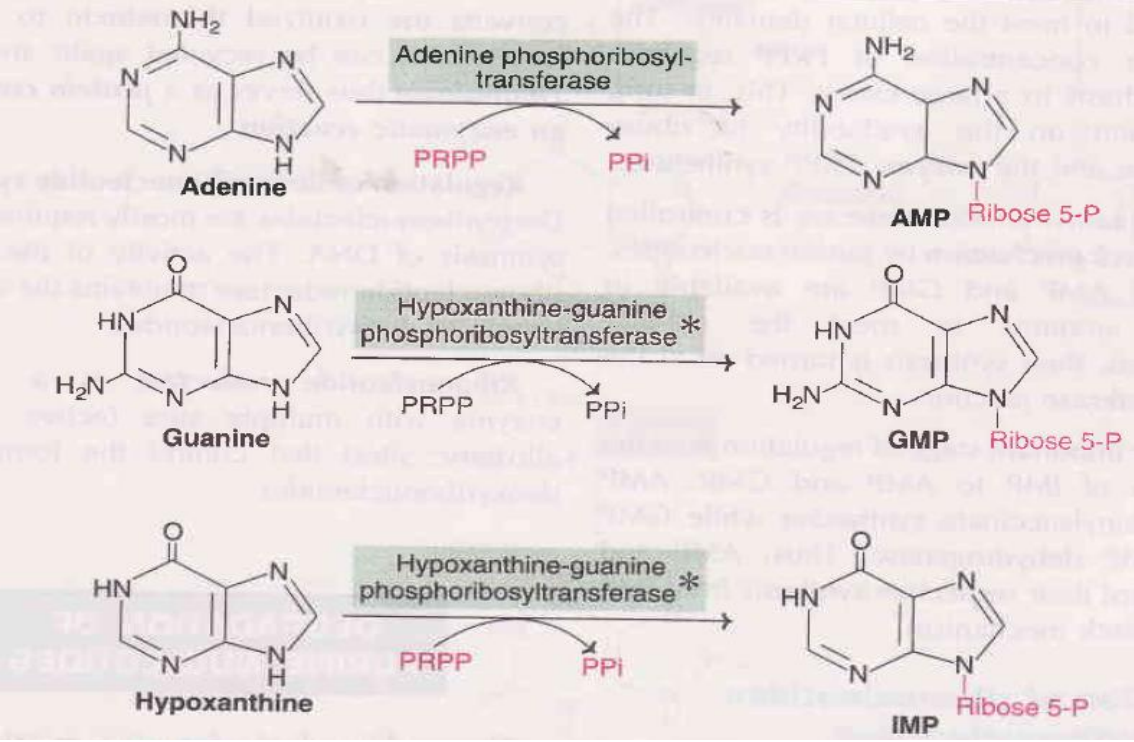
synthesis. The more important mechanism involves phosphoribosylation by PRPP of a free purine (Pu) to form a purine 5′-mononucleotide (Pu-RP).

$$Pu+PR-PP \rightarrow PRP+PPi$$

Two phosphoribosyl transferases then convert adenine to AMP and hypoxanthine and guanine to IMP or GMP. A second salvage mechanism involves phosphoryl transfer from ATP to a purine ribonucleoside (PuR):

$$PuR+ATP \rightarrow PuR-P+ADP$$

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



Salvage pathways of purine nucteotide synthesis

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

Regulation of purine nucleotide biosynthesis

- The purine nucleotide synthesis is well coordinated to meet the cellular demands. The intracellular concentration of PRPP regulates purine synthesis to a large extent. This, in

turn, is dependent on the availability of ribose S-phosphate and the enzyme PRPP synthetase.

- PRPP glutamyl amido transferase is controlled by a feedback mechanism by purine nucleotides. That is, if AMP and CMP are available in adequate amounts to meet the cellular requirements, their synthesis is turned off at the amidotransferase reaction.
- Another important stage of regulation is in the conversion of IMP to AMP and GMP. AMP inhibits adenylsuccinate synthetase while GMP inhibits IMP dehydrogenase. Thus, AMP and GMP control their respective synthesis from IMP by a feedback mechanism.
- Regulation of deoxyribonucleotide synthesis: Deoxyribonucleotides are mostly required for the synthesis of DNA. The activity of the enzyme ribonucleotide reductase maintains the adequate supply of deoxyribonucleotides.
- Ribonucleotide reductase is a complex enzyme with multiple sites (active site and allosteric sites) that control the formation of deoxyribo nucleotides.

BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

The catalyst for the initial reaction is cytosolic carbamoyl phosphate synthase II, a different enzyme from the mitochondrial carbamoyl phosphate synthase I of urea synthesis. Compartmentation thus provides two independent pools of carbamoyl phosphate. PRPP, an early participant in purine nucleotide synthesis, is a much later participant in pyrimidine biosynthesis.

The synthesis of pyrimidines is a much simpler process compared to that of purines. Aspartate, glutamine (amide group) and CO_2 contribute atoms in the formation of pyrimidine ring. Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate.

Salient features

Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalysed by cytosomal enzyme carbamoyl phosphate synthetase II (CPS II). CPS II is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase I (CPS I) is a mitochondrial enzyme which synthesizes carbamoyl phosphate from ammonia and CO_2 and, in turn urea. Prokaryotes have only one carbamoyl phosphate synthetase which is responsible for the biosynthesis of arginine and pyrimidines.

Carbamoyl phosphate condenses with aspartate to form carbamoyl aspartate. This reaction is catalysed by aspartate transcarbamoylase. Dihydroorotase catalyses the pyrimidine ring closure with a loss of H_2O.

Reactions of Pyrimidine Biosynthesis

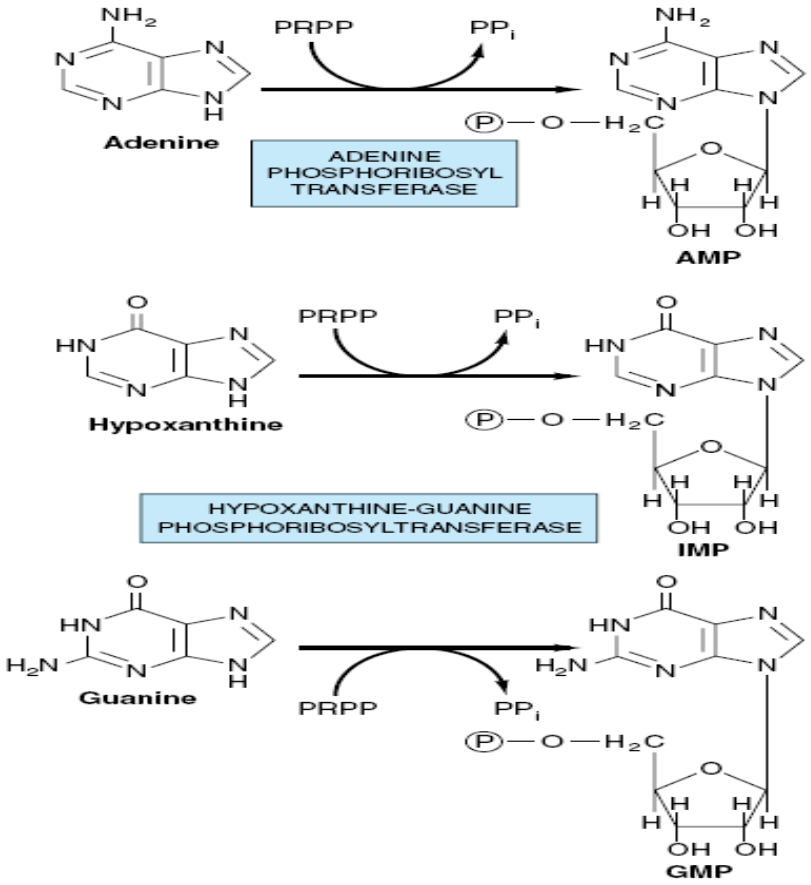
Five of the first six enzyme activities of pyrimidine biosynthesis reside on multifunctional polypeptides. One such polypeptide catalyzes the first three reactions and ensures efficient channeling of carbamoyl phosphate to pyrimidine biosynthesis. A second bifunctional enzyme catalyzes reactions 5 and 6.

Deoxyribonucleosides of Uracil & Cytosine

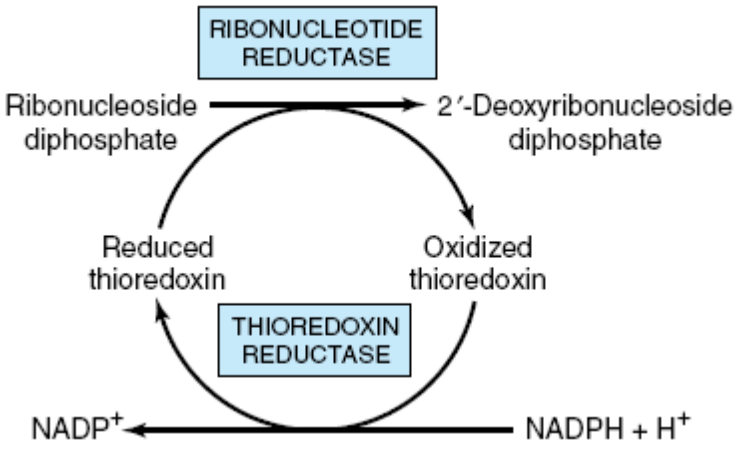
While mammalian cells reutilize few free pyrimidines, "salvage reactions" convert the ribonucleosides uridine and cytidine and the deoxyribonucleosides thymidine and deoxycytidine to their respective nucleotides. ATP dependent phosphoryl transferases (kinases) catalyze the phosphorylation of the nucleoside diphosphates 2′-deoxycytidine, 2′-deoxyguanosine, and 2′-deoxyadenosine to their corresponding nucleoside triphosphates. In addition, orotate phosphoribosyltransferase, an enzyme of pyrimidine nucleotide synthesis, salvages orotic acid by converting it to orotidine monophosphate (OMP).

Reduction of Dihydrofolate

The methylene group of N^5, N^{10}-methylene-tetrahydrofolate is reduced to the methyl group that is transferred, and tetrahydrofolate is oxidized to dihydrofolate. For further pyrimidine synthesis to occur, dihydrofolate must be reduced back to tetrahydrofolate, a reaction catalyzed by dihydrofolate reductase. Dividing cells, which must generate TMP and dihydrofolate, thus are especially sensitive to inhibitors of dihydrofolate reductase such as the anticancer drug methotrexate. Figure 4 and 5 shows the phosphoribosylation of adenine, hypoxanthine and guanine form AMP, IMP, and GMP, reduction of ribonucleoside diphosphates to 2′deoxyribonucleoside diphosphates respectively.



Phosphoribosylation of adenine, hypoxanthine and guanine to form AMP, IMP, and GMP, respectively.



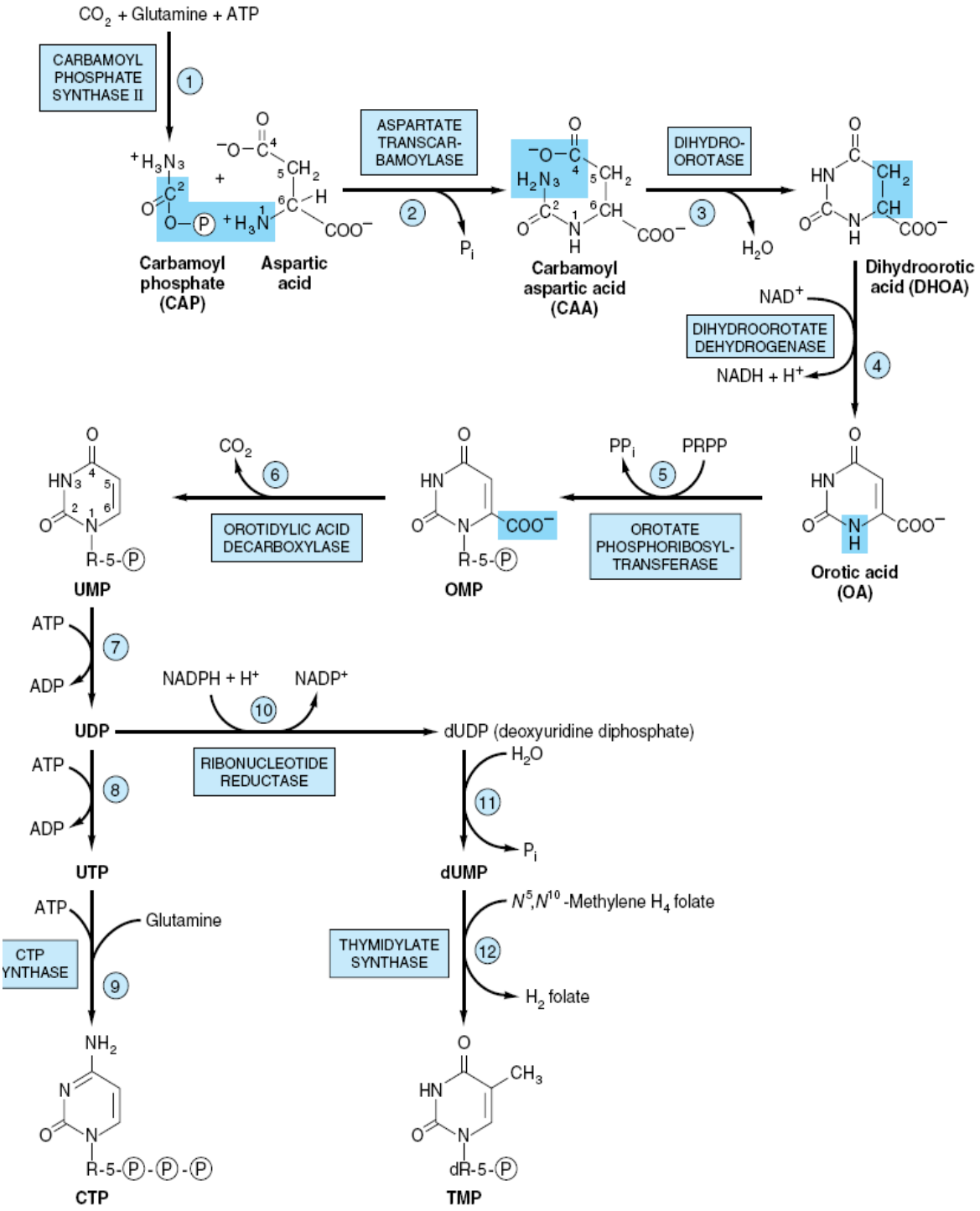
Reduction of ribonucleoside diphosphates to 2′-deoxyribonucleoside diphosphates

Certain Pyrimidine Analogs Are Substrates for Enzymes of Pyrimidine

Nucleotide Biosynthesis Orotate phosphoribosyltransferase converts the drug allopurinol to a nucleotide in which the ribosyl phosphate is attached to N-1 of the pyrimidine ring. The anticancer drug 5-fluorouracil is also phosphoribosylated by orotate phosphoribosyl transferase.

Pyrimidine Nucleotide Biosynthesis

Regulation of the rate of pyrimidine nucleotide synthesis in bacteria occurs in large part through aspartate transcarbamoylase (ATCase), which catalyzes the first reaction in the sequence and is inhibited by CTP, the end product of the sequence. The bacterial ATCase molecule consists of six catalytic subunits and six regulatory subunits. The catalytic subunits bind the substrate molecules, and the allosteric subunits bind the allosteric inhibitor, CTP. The entire ATCase molecule, as well as its subunits, exists in two conformations, active and inactive. When CTP is not bound to the regulatory subunits, the enzyme is maximally active. As CTP accumulates and binds to the regulatory subunits, they undergo a change in conformation. This change is transmitted to the catalytic subunits, which then also shift to an inactive conformation. ATP prevents the changes induced by CTP.



The biosynthetic pathway for pyrimidine nucleotides.

- The synthesis of pyrimidines is a much simpler process compared to that of purines. Aspartate, glutamine (amide group) and CO_2 contribute to atoms in the formation of

pyrimidine ring. Pyrimidine ring is first synthesized and then attached to ribose 5-phosphate. This is in contrast to purine nucleotides synthesis where in purine ring is built upon a pre-existing ribose 5-phosphate.

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- The three enzymes-CPS II, aspartate transcarbamoylase and dihydroorotase are the domains (functional units) of the same protein. This is a good example of a multifunctional enzyme.
- The next step in pyrimidine synthesis is an NAD^+ dependent dehydrogenation, leading to the formation of orotate. Ribose5 –phosphate is now added to orotate to produce orotidine monophosphate (OMP).
- This reaction is catalysed by orotate phosphoribosyl transferase, an enzyme comparable with HGPRT in its function. OMP undergoes decarboxylation to uridine mono-phosphate (UMP).
- By an ATP-dependent kinase reaction, UMP is converted to UDP which serves as a precursor for the synthesis of dUMP, dTMP, UTP and CTP.
- Ribonucleotide reductase converts UDP to dUDP by a thioredoxin-dependent reaction. Thymidylate synthetase catalyses the transfer of a methyl group from N^5, N^{1O}-methylene tetrahydrofolate to produce deoxythymidine monophosphate(dTMP).
- UDP undergoes an ATP-dependent kinase reaction to produce UTP. Cytidine triphosphate (CTP) is synthesized from UTP by amination. CTP synthetase is the enzyme and glutamine provides the nitrogen.

REGULATION OF PYRIMIDINE NUCLEOTIDE BIOSYNTHESIS

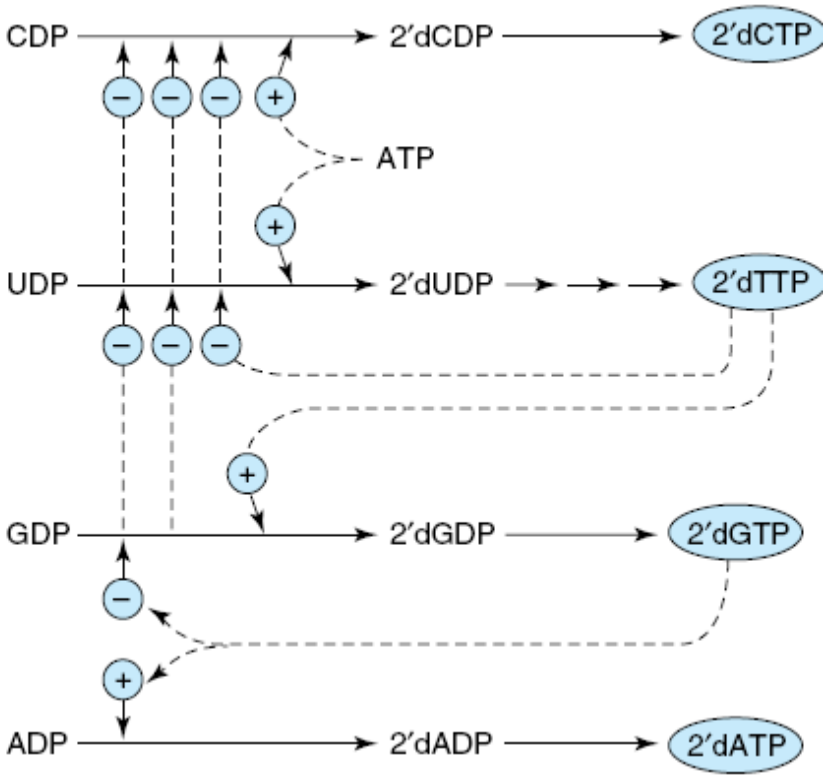
Gene Expression & Enzyme Activity Both Are Regulated

The activities of the first and second enzymes of pyrimidine nucleotide biosynthesis are controlled by allosteric regulation. Carbamoyl phosphate synthase II is inhibited by UTP and purine nucleotides but activated by PRPP. Aspartate transcarbamoylase is inhibited by CTP but

activated by ATP. In addition, the first three and the last two enzymes of the pathway are regulated by coordinate repression and derepression.

Biosynthesis of Purine & Pyrimidine Nucleotide

Purine and pyrimidine biosynthesis parallel one another mole for mole, suggesting coordinated control of their biosynthesis. Several sites of cross-regulation characterize purine and pyrimidine nucleotide biosynthesis. The PRPP synthase reaction, which forms a precursor essential for both processes, is feedback-inhibited by both purine and pyrimidine nucleotides.



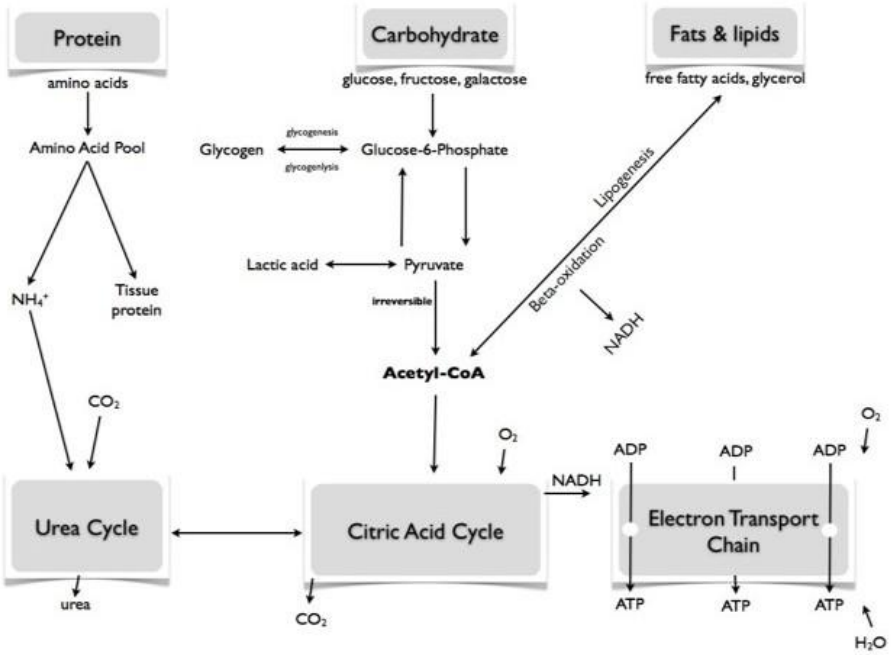
Regulation of the reduction of purine and pyrimidine ribonucleotides to their respective 2′-deoxyribonucleotides. Solid lines represent chemical flow. Broken lines show negative (–) or positive (+) feedback regulation.

INTER RELATIONSHIP OF CARBOHYDRATE, PROTEIN AND FAT METABOLISM

Cellular metabolism describes the process by which the products of digestion (intermediaries amino acids, fat derivatives and the hexoses/ monosaccharides fructose, galactose and glucose) are chemically modified to yield usable energy. The final common pathway of metabolism is the citric acid cycle, which accepts fragments of the intermediaries, releasing CO_2 and H^+ ions. Most catabolic energy is not immediately used but rather temporarily stored in

high-energy phosphate compounds as bonds between phosphoric acid residues. The most important of these is adenosine triphosphate (ATP). Hydrolysis of ATP to ADP yields energy directly for cellular and bodily function. **Acetyl-CoA** is another important energy source, and is molar equivalent to ATP in its ability to provide energy for cellular processes. ATP:

Carbohydrate Metabolism



- Most dietary carbohydrates are polymers of hexoses, primarily glucose, galactose and fructose.
- Glucose is stored in its phosphorylated form glucose-6-phosphate, the formation of which in muscles is catalyzed by **hexokinase**, and in the liver by **glucokinase**.
- Glucokinase is important because its activity is stimulated by insulin and it's activity reduced in starvation, and glucokinase has a stronger affinity for glucose than does hexokinase.
- Only hepatic (and insignificant amounts of renal) glycogen is accessible to the body as a glucose source. Glucose-6-phophatase is *only* found in the liver, converting Glc-6-P to glucose.
- Both adrenaline and glucagon stimulate hepatic phosphatase and so increase blood glucose levels by stimulating release of hepatic glucose.

- In this way the liver functions as the **hepatic glucostat,** releasing glucose when blood glucose levels are low and taking up glucose (stimulated by insulin) when blood glucose is elevated.

While carbohydrates can be converted to fats via glucose and acetylCoA, the textbooks tell us that the conversion of pyruvate to acetylCoA is an irreversible reaction, so supposedly **fats cannot be converted to significant amounts of glucose**. (though insignificant amounts of glycerol can be converted to glucose.)

Three main hexoses:

- Glucose – enters glycolysis
- Galactose – from the disaccharide lactose (comprises glucose and galactose)
- Fructose – metabolism is unaffected by insulin, so had been advocated as a suitable carbohydrate source for diabetics. However most fructose is simply metabolized by the liver and intestines. Readily converted into palmitic acid.

Lipid Metabolism

- Triglycerides are the largest dietary fat source, comprising three fatty acids joined to a glycerol backbone.
- Hydrolysis of triglycerides in the small intestine results in Free Fatty Acids & glycerol. Absorbed into enterocytes and packaged with cholesterol into chylomicrons, excreted into the lymph system, and subsequently reach the circulation via the thoracic duct and the left subclavian vein.
- Glycerol is converted to glycerol-3P which enters the glycolytic pathway and can then be converted to glucose.
- **Beta-oxidation** of fatty acids produces **Acetyl-CoA**.

Beta oxidation involves:

- Activation of the fatty acids in the cytosol through reactions with ATP and Coenzyme A.
- Carriage of FFA into mitochondria by carrier-protein **'carnitine'**.
- Beta oxidation in the mitochondrial matrix
- AcetylCoA enters Krebs cycle, combing with OA to form citrate and subsequently release CO_2 and H^+.

- Produces **lots** of energy, with 1 stearic acid molecule (-> 9 acetyl CoA) producing 146 ATPs.
- In the liver only some of the acetylCoA is used in Krebs, with a large proportion:
  - 2 AcetylCoA molecules condense to form acetoacetate
  - Some acetoacetate -> β-hydroxybutyrate and then a very small amount β - hydroxybutyrate -> acetone.
- Acetoacetate, beta-hydroxybutyrate and acetone are ketone bodies.
- These ketone bodies are transported in the blood to tissues, where the reverse reaction occurs, releasing AcetylCoA.
- **Oxaloacetate** is required to bind AcetylCoA for its use in the Krebs Cycle, which becomes deficient if carbohydrate metabolism ceases (as occurs in a big switch to FFA metabolism), hence causing ketosis due to excess acetyl-CoA condenses to acetoacetyl-CoA.

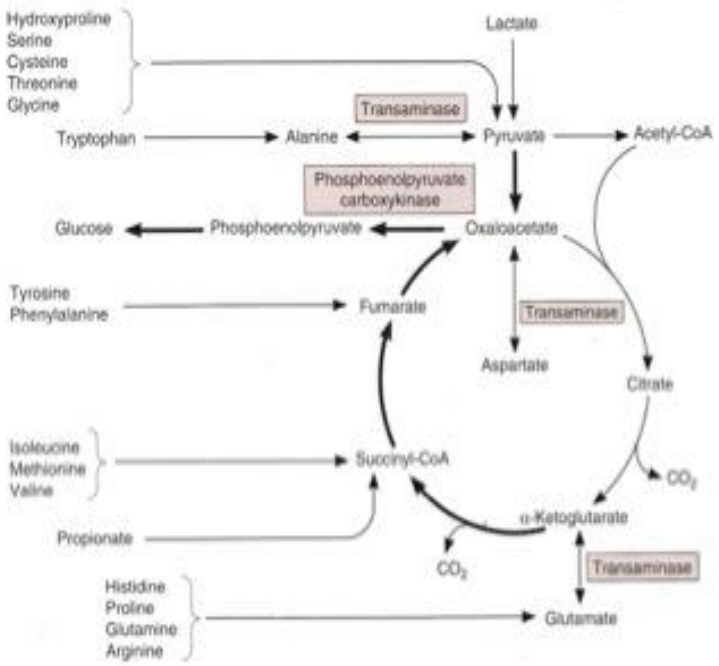
Ketosis occurs in three conditions:

1. Fasting or Starvation
2. Diabetes mellitus
3. High-fat/protein low-carbohydrate diets

Fatty acids can be readily used as an energy source by most body tissues, except: the brain, erythrocytes and the adrenal medulla.

Protein Metabolism

- Proteins are chains of amino acids linked by peptide bonds between amino and carboxyl groups. There is much variation and complexity in different protein structures.
- Most proteins are digested in the gastrointestinal tract and absorbed as amino acids. These amino acids, combined with those resulting from turnover of endogenous body protein, are termed the**amino acid pool**.
- Interconversion between amino acids and products of fat and carbohydrate metabolism at the level of the common metabolic pool revolve around amino group transfer (**transamination**), removal (**deamination**) or formation.



Integration of Major Metabolic Pathways of Energy Metabolism

Though metabolism of each of major food nutrients, viz. carbohydrates, lipids and proteins have been considered separately for the sake of convenience, *it actually takes place simultaneously* in the intact animal and are closely interrelated to one another. The metabolic processes involving these three major food nutrients and their interrelationship can be broadly divided into three stages

1st stage: Stage of hydrolysis to simpler units

2nd stage: Preparatory stage

3rd stage: Oxidative stage–Aerobic final (TCA Cycle).

1st Stage

Stage of Hydrolysis to Simpler Units

- The complex polysaccharides, starch/glycogen are broken down to glucose; and disaccharides are hydrolysed to monosaccharides in GI tract by various carbohydrate-splitting enzymes present in digestive juices.
- Similarly, principal lipids, triacylglycerol (TG) is hydrolysed to form FFA and glycerol.
- Proteins are hydrolysed by proteolytic enzymes to amino acids.

The above is the prelude to either further synthesis of new substances or for their oxidation. Very little of energy is produced in this hydrolytic phase and it is dissipated away as heat. There is no storage of energy at this stage.

2nd Stage

Preparatory Stage

- The monosaccharide glucose runs through the glycolytic reactions to produce the 3-C keto acid pyruvic acid (PA) in the cytosol, which in turn is transported to mitochondrion where it undergoes oxidative decarboxylation to produce 2-C compound "acetyl-CoA" ("active" acetate).
- The glycerol of fat, either goes into formation of glucose (gluconeogenesis) or by entering the same glycolytic pathway through the triose-P, forms PA and then finally 2-C compound "acetyl-CoA"
- The fatty acids undergo principally β-oxidation and form several molecules of "acetyl-CoA".
- The amino acids are deaminated/and/or transaminated first and the C-skeleton is metabolised differently from amino acid to amino acid
- In the case of amino acids, viz. Glycine, Alanine, Serine, Cysteine/Cystine and threonine when catabolised form pyruvic acid (PA) similar to carbohydrates and is finally converted to 'Acetyl CoA'
- In the case of amino acids, viz. Glutamic acid, Histidine, Proline and OH-proline, Arginine and Ornithine produces α-ketoglutaric acid when catabolised and thus they enter the TCA cycle.
- Yet a few others like Leucine, Phenyl alanine, Tyrosine and Isoleucine yield acetate or acetoacetate, the latter can be converted to "acetyl- CoA".

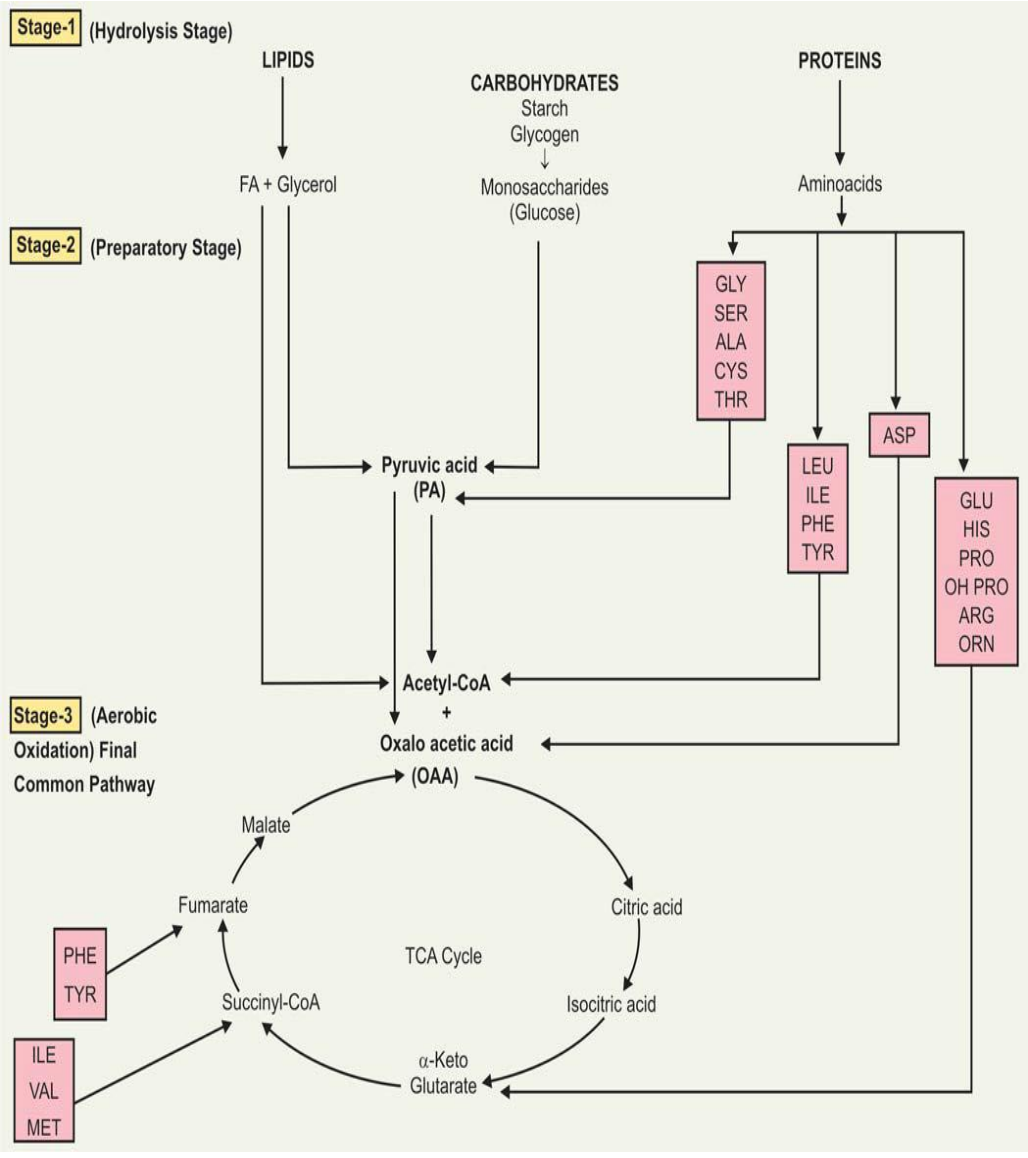
During the second stage (glycolysis, β-oxidation, etc.) relatively small amount of energy is produced and this is stored as ATP.

3rd Stage

Oxidative Stage: Aerobic Final (TCA Cycle)

In presence of oxygen, acetyl-CoA is oxidised to CO_2 and H_2O by common final pathway TCA cycle.

The carbohydrates, lipids and proteins all form acetate or some other intermediates like oxaloacetate (OAA), α-ketoglutarate, succinyl-CoA, or fumarate, which are all intermediates of TCA cycle. Having gainedentry into the TCA cycle at any site, two of carbons of "citrate" constituting an acetate moiety are oxidised finally to CO2 and H2O and the energy of oxidation by the electron transport chain is captured as energy-rich PO4 – ATP mostly. ***This stage yields the largest amount of energy*** of all three stages. Thus, the pathways are similar to a large extent and identical in the final stage of oxidation of the metabolites, whether derived from carbohydrates, lipids or proteins.



INTERCONVERSION BETWEEN THE THREE PRINCIPAL COMPONENTS

I. Carbohydrates

1. Carbohydrates can form lipids:

Through formation of: (a) α-glycero-P from glycerol or di-hydroxy acetone- P (from glycolysis) which is necessary for Triacyl glycerol (TG) and (b) FA from acetyl-CoA-extramitochondrial *de novo* synthesis.

2. Carbohydrates can form non-essential amino acids:

Through amination of α-ketoacids, viz. pyruvic acid (PA), oxaloacetic acid (OAA) and α-ketoglutarate to form amino acids alanine, aspartate and glutamate respectively.

II. Fats

- Fatty acids can be converted to some amino acids by forming the dicarboxylic acids like malic acid, oxalo acetic acids and α-ketoglutarate.
- Fatty acid carbon may theoretically be incorporated into carbohydrates by the acetate running through TCA cycle. But there is no net gain in carbohydrates, since two carbons, equivalent of acetate are oxidized in the cycle.
- However acetate can form glucose by running through the glyoxylate cycle.
- Acetone, one of the ketone bodies may be glucogenic. Acetone can be converted to acetol-P which in turn can produce propanediol-P. Propanediol-(P) is glucogenic.

III. Proteins

Proteins can form both carbohydrates and lipids through the glucogenic and ketogenic amino acids.

Regulation and Control of the Reactions

The ratio of ATP/AMP of the cells/or tissues seems to decide the extent of its aerobic metabolism.

(a) Inhibition: If the ratio is high (low AMP or ADP level), this will have certain inhibitory effects of certain enzymes of glycolytic-TCA cycle.

A high level of ATP and low level of AMP will inhibit the enzyme *phosphofructokinase* of glycolytic pathway and thereby inhibit glycolysis. As a result there is accumulation of hexose-P which interacts with UTP to form UDP-G and proceeds to increased glycogen synthesis. G-6-P will also be channelised to HMP-shunt leading to increased formation of NADPH which will

participate in reductive synthesis, like FA synthesis which will be increased. The converse happens with low ATP and high AMP levels.

1. Increased ATP/ADP ratio will stimulate *PDH-kinase* which in turn converts dephosphorylated *active PDH (pyruvate dehydrogenase* complex) to 'inactive' phosphorylated PDH inhibiting the oxidative decarboxylation of pyruvic acid (PA).
2. High ATP/AMP ratio, also lowers the activity of the enzymes *Isocitrate dehydrogenase* (ICD) of TCA cycle resulting in accumulation of citrate. The oxidation in TCA cycle decreases and ATP production falls.

(b) Stimulation: Increased citric acid levels stimulate the enzyme *acetyl-CoA carboxylase.* Increased activity of acetyl-CoA carboxylase converts acetyl-CoA to malonyl- CoA, the first step in extramitochondrial *de novo* FA synthesis. Thus, the acetyl-CoA, in the presence of adequate stores of ATP and low AMP levels, is diverted to the synthesis of fats. The reverse set of conditions operates when the ATP/ AMP ratio is low.

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COIMBATORE - 21
DEPARTMENT OF BIOCHEMISTRY
III B.Sc BIOCHEMISTRY
BATCH: 2015 - 2018

PART A (20 X 1 = 20 MARKS) - Online MCQ questions

SUBJECT: METABOLIC PATHWAYS **SUBJECT CODE: 15BCU501** **UNIT: V**

S. No	Question	Option A	Option B	Option C	Option D	Answer
	Hypoxanthine and ribose constitute	Adenosine	Inosine	Guanosine	Cytidine	Inosine
	Thymidylate synthetase	catalyses the methylation of UTP to dTTP	uses NADPH as a cofactor	is inhibited by fluorodeoxy uridylate	uses a protein called thioredoxin	catalyses the methylation of UTP to dTTP
	N – 3 of purine is donated by	Glutamine	Glutamic acid	Tetra hydro folic acid	Folic acid	Glutamine
	Thymidylate synthetase requires	N^5 methyl tetrahydrofolate	N^{10} formyl tetrahydrofolate	$N^5 – N^{10}$methylene tetrahydrofolate	N^5 formyl tetrahydrofolate	$N^5 – N^{10}$methylene tetrahydrofolate
	Which one of the following compounds act as ribose phosphate donor?	ATP	PRPP	AMP	ADP	PRPP
	In salwage pathway, one of the following does not take place	purine to purine nucleotide	ribose – 5 – phosphate and ATP to purine nucleotide	purine ribonucleoside to purine nucleotide	purine deoxy ribonucleoside to purine nucleotide	ribose – 5 – phosphate and ATP to purine nucleotide
	The sources of N1 – atoms of purine ring are	Aspartate	Glutamate	Glycine	Ammonia	Aspartate
	Inosinic acid is the biological precursor of	cytosine and uric acid	adenylic acid and guanylic acid	orotic acid and uridylic acid	uracil and thymidine	adenylic acid and guanylic acid
	The source of the nitrogen atom N7 of the purine ring are	aspartate	glutamate	glycine	ammonium ion	glycine
	The following are the end products of pyrimidine catabolism	ammonia and carbondioxide	α-alanine	β-aminosobutyrate	β-alanine	ammonia and carbondioxide
	Carbon 6 of purine skeleton comes from	respiratory CO_2	1-carbon carried by folate	betaine	methionine	respiratory CO_2
	The amino acid which is not involved in purine biosynthesis	glycine	methionine	aspartate	glutamine	methionine
	Pyrimidine metabolism yields	uric acid	creatinine	allantoin	Xanthine	creatinine
	Methotrexate an anticancer drug inhibits	dihydro folate reductase	CTP synthase	thymidylate synthase	carbamoyl phosphate synthase	dihydro folate reductase
	Phosphoribosyl pyro phosphate synthase	requires ATP and Mg	inhibited by AMP and ADP	inhibited by GMP and GDP	Inhibited by NADH and NADPH	requires ATP and Mg
	Dihydroorotic acid is involved in the biosynthesis of	purine nucleotide	Riboflavin	pyrimidine nucleotide	Pyridoxine	pyrimidine nucleotide
	Purine catabolism results in the formation of	urea	uric acid	creatinine	xanthine	uric acid
	All statements about 5 fluoro uracil are true except	analog of uracil	inhibits dihydrofolate reductase	has anti neoplastic activity	inhibits pyrimidine metabolism	inhibits dihydrofolate reductase
	Nucleotides are further hydrolysed to free purine and pyrimidine bases by	nucleotidases	nucleosidases	proteases	ribonuclease	nucleotidases
	A genetic lack of hypoxanthan – guanine phosphoribosyl transferase activity results in a set of symptoms called	Guanine deaminase deficiency	Gout	Lesch-Nyhan Syndrome	Xanthine oxidase Deficiency	Lesch-Nyhan Syndrome
	Conversion of IMP to XMP is inhibited by	GMP	AMP	ADP	GTP	GMP
	The chemical name for 2 amino 6 oxy purine is said to be	adenine	Guanine	xanthine	hypoxanthine	guanine
	The catabolic product of pyrimidine nucleotide is	urea	uric acid	allantoin	β aminoisobutyrate	urea
	Allopurinol inhibits	xanthine oxidase	urate oxidase	adenine deaminase	guanine oxidase	xanthine oxidase
	The conversion of xanthine to uric acid is catalysed by	urate oxidase	xanthine oxidase	guanine deaminase	adenine deaminase	xanthine oxidase
	Malonate is a competitive inhibitor of	alcohol dehydrogenase	lactate dehydrogenase	Malate dehydrogenase	succinate dehydrogenase	succinate dehydrogenase
	Nitrogen for the amination of 5- phosphor ribosyl 1-pyro phosphate is derived from	ammonia	Aspartate	glutamine	glycine	glutamine
	Lesch-Nyhan syndrome, the sex linked, recessive absence of HGPRTase, may lead to	Compulsive self destructive behaviour with elevated levels of urate in serum	Hypouricemia due to liver damage	Failure to thrive and megaloblastic anemia	Protein intolerance and hepatic encephalopathy	Compulsive self destructive behaviour with elevated levels of urate in serum
	Inherited deficiency of adenosine deaminase causes	Hyperuricaemia and gout	Mental retardation	Immunodeficiency	Dwarfism	Immunodeficiency
	Complete absence of hypoxanthine guanine phospharibosyl transferase causes	Primary gout	Immunodeficiency	Uric acid stones	Lesh-Nyhan syndrome	Lesh-Nyhan syndrome
	The major catabolic product of pyrimidines in human is _____	β-Alanine	Urea	Uric acid	Guanine	β-Alanine
	In humans, the principal break down product of purines is	NH3	Allantin	Alanine	Uric acid	Uric acid
	Inheritance of primary gout is _____	Autosomal recessive	Autosomal dominant	X-linked recessive	X-linked dominant	X-linked recessive
	Hypouricaemia can occur in _____	Xanthine oxidase deficiency	Psoriasis	Leukaemia	Gauche's disease	Xanthine oxidase deficiency
	Synthesis of DNA is also known as _____	Duplication	Replication	Transcription	Translation	Replication
	DNA does not contain	Thymine	Adenine	Uracil	Deoxyribose	Uracil
	The first member of cytochrome accepting electron in the respiratory chain is _____	cytochrome a	cytochrome b	cytochrome c1	cytochrome c	cytochrome b
	A high ATP/AMP ratio will inhibit which of the following enzyme?	Glyceraldehyde-3-P dehydrogenase	Pyruvate kinase	Enolase	Phosphofructokinase	Phosphofructokinase
	Increased citric acid levels in the blood will stimulate which of the enzyme?	Transketolase	Pyruvate carboxylase	Acetyl-CoA carboxylase	Enolase	Acetyl-CoA carboxylase
	which cycle is consider as a central core of metabolism?	Glycolysis	TCA cycle	HMP shunt	Urea cycle	TCA cycle
	The four nitrogen atoms of purines are derived from _____	Urea and ammonia	Ammonia, glycine and glutamate	Glycine, ammonia and aspartate	Aspartate, glutamine and glycine	Aspartate, glutamine and glycine
	A drug which prevents uric acid synthesis by inhibiting the enzyme xanthine oxidase is _____	Aspirin	Allopurinol	Colchicine	Probenecid	Allopurinol
	Glycine contributes to the following C and N of purine nucleus _____	C-1, C-2, and N-7	C-8, C-6 and N-9	C-4, C-5 and N-7	C-3, C-4 and N-1	C-4, C-5 and N-7
	Inosinic acid is the biological precursor of _____	Cytosine and uric acid	Adenylic acid and guanylic acid	Orotic acid and uridylic acid	Adenosine and thymidine	Adenylic acid and guanylic acid
	The probable metabolic defect in gout is _____	A defect in excretion of uric acid by kidney	An overproduction of pyrimidines	An overproductin of uric acid	Rise in calcium leading to deposition of calcium urate	An overproductin of uric acid
	Purine base is _____	Guanine	Cytosine	Thymine	Uracil	Guanine
	Inosinic acid is a _____	Purine	Pyrimidine	Nucleoside	Nucleotide	Nucleotide
	Adenosine is _____	Nucleotide	Nucleoside	Purine	Pyrimidine	Nucleoside
	A key substance in the committed step of pyrimidine biosynthesis is _____	Ribose-5'-phosphate	Carbamoyl phosphate	Thiouracil	Glutamine	Carbamoyl phosphate
	In most mammals, except primates, uric acid is metabolized by _____	Reduction to NH3	Hydrolysis to ammonia	Oxidation to allantoin	Hydrolysis to allantoin	Oxidation to allantoin
	5-phosphoribosyl-1-amine synthesis inhibited by _____	6-Mercaptopurine	6-Azauracil	5-Fluorodeoxyuridine	Methotrexate	Methotrexate
	AMP synthesis is inhibited by	Azaserine	6-Mercaptopurine	Methotrexate	6-Azauracil	6-Mercaptopurine
	Two Nitrogen of the pyrimidine ring are obtained from	Glutamine and carbamoly-P	Glutamate and ammonia	Aspartate and carbamoly-P	Aspartate and ammonia	Aspartate and carbamoly-P
	Which statement is NOT true about Lesch-Nyhan syndrome?	Mental retardation	Inheritence is autosomal recessive	Elevated levels of uric acid in blood	Genetic deficiency of the enzyme HGPRT	Inheritence is autosomal recessive
	Synthesis of GMP from IMP requires _____	Ammonia, NAD^+, ATP	Glutamine, NAD^+, ATP,	Ammonia, GTP, $NADP^+$	Glutamine, UTP, $NADP^+$	Glutamine, NAD^+, ATP,
	Which pathway is correct for catabolism of purines to form uric acid?	Inosinate → adenylate → xanthine → hypoxanthine → uric acid.	Guanylate → inosinate → xanthine → hypoxanthine → uric acid	Adenylate → Inosinate → xanthine → hypoxanthine → uric acid	Adenylate → inosinate → hypoxanthine → Uric acid	Adenylate → inosinate → hypoxanthine → Uric acid
	Gout is a metabolic disorder of catabolism of _____	Pyrimidine	Purine	Alanine	Phenylalanine	Purine
	A substrate for enzymes of pyrimidine nucleotide biosynthesis is _____	Allopurinol	Tetracylin	Chloramphenicol	Puromycin	Allopurinol
	UTP is converted to CTP by _____	Methylation	Isomerisation	Amination	Reduction	Amination

	In humans purine are catabolised to uric acid due to lack of the enzyme _____	Urease	Uricase	Xanthine oxidase	Guanase	Uricase
	PRPP glutamyl amidotransferase, the first enzyme uniquely committed to purine synthesis is feed back inhibited by _____	AMP	IMP	XMP	CMP	AMP
	Conversion of inosine monophosphate to xanthine monophosphate is catalysed by _____	IMP dehydrogenase	Formyl transferase	Xanthine-guanine phosphoribosyl transferase	Adenine phosphoribosyl transferase	IMP dehydrogenase
	Dietary purines are catabolised in _____	Liver	Kidneys	Intesitnal mucosa	Pancrease	Intesitnal mucosa
	Orotic aciduria can be controlled by _____	Oral administration of orotic acid	Decreasing the dietary intake of orotic acid	Decreasing the dietary intake of pyrimidines	Oral administration of uridine	Oral administration of uridine

Reg. No.....

[15BCU501]

KARPAGAM UNIVERSITY

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(For the candidates admitted from 2015 onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2017

Fifth Semester

BIOCHEMISTRY

METABOLIC PATHWAYS

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 8 = 40 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. Briefly explains about the mechanism of oxidative phosphorylation.
Or
b. Write an account on enzymes and co enzyms involved in redox reaction.

22. a. Explain the HMP shunt pathways.
Or
b. Describe the glycolysis pathway.

23. a. Write an account on the biosynthesis of cholesterol.
Or
b. Write a notes on plasma lipoproteins

24. a. Explain about urea cycle.
Or
b. Briefly explain about deamination and transamination reactions.

25. a. Describe the pathway of purine biosynthesis
Or
b. Explain about the inter conversion of carbohydrates and proteins.

Reg. No.....

[14BCU501]

KARPAGAM UNIVERSITY

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(For the candidates admitted from 2014 onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2016

Fifth Semester

BIOCHEMISTRY

METABOLIC PATHWAYS

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 8 = 40 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a) Write detail account on Catabolism and its pathways.
Or
b) Elaborate the mechanism of oxidative phophorylation.

22. a) Explain the steps involved in Glycolysis.
Or
b) Give account on HMP Shunt.

23. a) Discuss on biosynthesis of saturated fatty acid.
Or
b) Write notes on the formation of ketone bodies.

24. a) Discuss the steps involved in disposal of ammonia.
Or
b) Write account on catabolism of amino acids.

25. a) Explain the biosynthesis of pyrimidine.
Or
b) Elaborate TCA cycle and its pathways.

Reg. No.....

[12BCU501]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

B.Sc. DEGREE EXAMINATION, NOVEMBER 2014

Fifth Semester

BIOCHEMISTRY

METABOLIC PATHWAYS

Time: 3 hours Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. What constitutes intermediary metabolism?
2. How many types of oxido-reductases involve in oxidative process? Give one example for each type?
3. What is an anabolic pathway and give one example?
4. Comment on the fate of absorbed carbohydrates.
5. How the glucose is utilized in the human body?
6. What are the alternate pathways of carbohydrate metabolism? Specify their role.
7. State the biomedical importance of blood lipids.
8. What is β-oxidation?
9. Draw the structure of the phospholipid.
10. What is transamination? Give an example?
11. Define amino acid catabolism.
12. Comment on the disposal of ammonia.
13. List importances of purine and pyrimidine biosynthesis.
14. What is salvage pathway? Give an example.
15. What are the inhibitors of purine biosynthesis?

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a) Discuss the schematics of glycolytic pathway with rate limiting steps.

Or

b) Explain the gluconeogenetic process with rate limiting steps.

17. a) Explain the oxidation of fatty acids with odd numbers of carbon atoms and illustrate the biosynthesis of saturated fatty acids.

Or

b) Draw the schematics of cholesterol and gylcolipid biosynthesis.

18. a) Explain in detail the transamination and deamination of amino acids.

Or

b) How ammonia is transported for disposal through the urea cycle?

19. a) Explain the biosynthesis of purines and pyrimidines (salvage pathway) through a flow chart.

Or

b) Elucidate the catabolism of purine and pyrimidine bases.

20. **Compulsory : -**

Elucidate the role of uncouplers of oxidative phosphorylation in deciphering the organization of respiratory chain in the mitochondria.
