KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2018 onwards)

DEPARTMENT OF BIOCHEMISTRY

STAFF NAME : Dr.K.DEVAKI
SUBJECT : REGULATION OF METABOLIC PATHWAYS
SEMESTER : II
SUBJECT CODE : 18BCP201 **CLASS : I M.Sc.(BC)**

UNIT I:

INTRODUCTION TO CONTROL OF ENZYME ACTIVITY

Allosteric interaction; Reversible covalent modification; proteolytic action; control of amount of enzyme; control of rates of enzyme degradation; feed back inhibition; feed forward stimulation. Role of compartmentation. Elucidation of Metabolic pathways- Single-and Multi-step pathways. Experimental approaches to study the metabolism- using metabolic inhibitors and isotopes.

UNIT II:

CARBOHYDRATE METABOLISM

An overview of Glycolysis and Gluconeogenesis. Role of LDH. Regulation of Glycolysis and Gluconeogenesis-Reciprocal control of Glycolysis and Gluconeogenesis, TCA cycle- steps, regulation at branch points; Glycogen Metabolism: Overview of glycogenesis and glycogenolysis. Reciprocal control of glycogenesis and glycogenolysis. Alternative pathways of metabolism-HMP shunt, Entner- doudoroff pathway, glucoronate and Glyoxalate pathway, cori cycle. Hormonal regulation of fuel metabolism; Metabolic disorders-Diabetes mellitus and insipidus.

UNIT III:

LIPID METABOLISM

An overview of fatty acid synthesis and degradation, Regulation of fatty acid synthesis- control of acetyl CoA carboxylase and fatty acid synthetase complex; Reciprocal control of fatty acid synthesis and degradation. Biosynthesis of triacyl glycerol, phosphatidyl choline, phosphotidyl ethanolamine and sphingomyelin and their regulation. Synthesis and degradation of cholesterol and its regulation. Metabolism of prostaglandins-COX and LOX pathways. Metabolic fate of VLDL, LDL and HDL. Obesity and regulation of body mass. Metabolic disorders-Atherosclerosis, Hyper and hypo lipoproteinemia.

UNIT IV:

AMINO ACID METABOLISM

Regulation of synthesis of pyruvate, serine, glutamate, aspartate, aromatic and histidine family of amino acids (Flow chart only) . Key role of glutamate dehydrogenase and glutamine synthetase in nitrogen metabolism and their allosteric regulations. Amino acid degradation- Oxidative deamination, Non oxidative deamination,decarboxylation and transamination. Ammonia formation and disposal- urea cycle and its regulation. Catabolism of carbon skeleton of amino acids.Biosynthesis of heme (porphyrin) and its regulations. Molecules derived from amino acids. Metabolic disorders- Alkaptonuria, phenyl ketonuria.

UNIT V:

NUCLEIC ACID METABOLISM

De novo synthesis of purine and its regulation – Role of PRPP amino transferase. De novo synthesis of pyrimidine and its regulation – Role of aspartate carbomyl transferase. Regulation of deoxy ribonucleotides by activators and inhibitors. Intergration of metabolism. Metabolism during starvation. Tissue specific metabolism- Metabolic profile of major organs- Brain, Muscle, Liver and Adipose tissue. Metabolic disorders- Gout, SCID.

SUGGESTED READINGS

1. Lehninger, L., Nelson, D.L., and Cox, M.M., (2012). Principles of Biochemistry, 6th edition WH Freeman and Company, New York.
2. Murray, R.K., Bender, D.A., Botham, K.M., and Kennelly, P.J., (2012). Harper's illustrated Biochemistry, 29th Edition. McGraw-Hill Medical. London.
3. Donald Voet and Judith Voet ,2004. Biochemistry, John Wiley and Sons,. 2ndEedition. New York
4. Leubert Stryer, 2009. Biochemistry, W.H. Freeman and Company. New York.
5. Pamila C. Champ and Richard A. Harvey ,2008. Biochemistry, Lipponcott Company, Philadelphia.
6. Smith. 2003. Principles of Biochemistry, McGraw– Hill International Book Company, London.
7. Zubay, G., (2009). Biochemistry, W.C Brown Publishers, Saunders and Company, Philadelphia.

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Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Dr.K.DEVAKI
SUBJECT NAME: Regulation of Metabolic Pathways SUB.CODE:18BCP201
SEMESTER:II CLASS: I M.Sc (BC)

S. No	Duration of period	Topics covered	Books referred	Page No	Web page referred
		UNIT-I			
1	1	**Introduction to control of enzyme activity-** Allosteric interaction, Reversible covalent modification;	S1 S2 S2	225-227 237-243 101-102	-
2	1	proteolytic action; control of amount of enzyme; control of rates of enzyme degradation	S4 S5	247-251 56-57	-
3	1	feed back inhibition; feed forward stimulation.	S4	727-728	-
4	1	Role of compartmentation.	S2	97-98	-
5	1	Elucidation of Metabolic pathways- Single pathway, Multi-step pathways.	S6	236-238	-
6	1	Experimental approaches to study the metabolism- using metabolic inhibitors	S3 S6	559-560 238-239	-
7	1	Experimental approaches to study the metabolism- using isotopes.	S3	562-565	-
8	1	Revision and QP discussion	-	-	-
		Total No of Hours Planned For Unit I : 08			
		UNIT-II			
1	1	**Carbohydrate Metabolism:** An overview of Glycolysis, Regulation of Glycolysis, Role of LDH	S1 S2	575-577 172-180	-
2	1	An overview of Gluconeogenesis and its regulation, Reciprocal control of Glycolysis and Gluconeogenesis	S2 S1	190-196 580-583	-
3	1	TCA cycle- steps, regulation at branch points	S6	299-301	-
4	1	Glycogen Metabolism: Overview of glycogenesis, Overview of glycogenolysis	S2	181-183	-
5	1	Reciprocal control of glycogenesis and glycogenolysis	S2 S4	183-187 590-597	-

6	1	Alternative pathways of carbohydrate metabolism- HMP shunt, ED pathway, glucoronate and glyoxalate pathway	S2	201-210	-
7	1	Hormonal regulation of fuel metabolism	S6	570-573	-
8	1	Metabolic disorders-Diabetes mellitus and insipidus.	-	-	W2
9	1	Revision and QP discussion	-	-	-
		Total No of Hours Planned For Unit II: 09			
		UNIT-III			
1	1	**Lipid metabolism:** An overview of fatty acid synthesis, Regulation of fatty acid synthesis-control of acetyl CoA carboxylase and fatty acid synthetase complex	S1 S4 S1	212-220 614-615 795-798	-
2	1	**Lipid metabolism:** An overview of fatty acid degradation, Reciprocal control of fatty acid synthesis and degradation	S1 S6	637-639 427-432	-
3	1	Biosynthesis of triacyl glycerol, phosphatidyl choline, phosphotidyl ethanolamine, sphingomyelin and their regulation	S6	441-452	-
4	1	Synthesis and degradation of cholesterol and its regulation	S1 S5	816-820 209-210	-
5	1	Metabolism of prostaglandins-COX and LOX pathway, Metabolic fate of VLDL,LDL and HDL	S2	250-258	-
6	1	Obesity and regulation of body mass.	S1	910-916	-
7	1	Metabolic disorders- Atherosclerosis, Hyper and hypo lipoproteinemia Metabolic disorders-	- -	- -	W31 W32
8	1	Revision and discussion of possible question	-	-	-
		Total No of Hours Planned For Unit III: 08			
		UNIT-IV			
1	1	**Amino acid metabolism:** Regulation of synthesis of pyruvate, serine, glutamate, aspartate family of amino acids	S2	287-300	-
2	1	Synthesis and regulation of aspartate family of amino acids	S1	841-845, 851-852	-
3	1	Synthesis and regulation of aromatic family of aminoacids	S2	845-850, 852-853	-
4	1	of synthesis of aromatic family of aminoacids	S2	852-853	-
5	1	Key role of glutamate dehydrogenase and glutamine synthetase in nitrogen metabolism and its regulation	S1 S4	834-846 728-729	-
6	1	Amino acid degradation- Oxidative and non	S3	985-990	-

		oxidative deamination, decarboxylation and transamination			
7	1	Urea cycle and its regulation, Biosynthesis of heme (porphyrin) and its regulations	S4	730-735	-
8	1	Molecules derived from aminoacids.	S6	526-530	-
9	1	Metabolic disorders- Alkaptonuria, phenyl ketonuria.	S2	310-311; 312-315	-
		Total No of Hours Planned For Unit IV: 09			
UNIT-V					
1	1	**Nucleic acid metabolism:** De novo synthesis of purine and its regulation – Role of PRPP amino transferase.	S2	363-368	-
2	1	De novo synthesis of pyrimidine and its regulation – Role of aspartate carbomyl transferase.	S2	369-372	-
3	1	Regulation of deoxy ribonucleotides by activators and inhibitors.	S1	869-873	-
4	1	Intergration of metabolism **Metabolism during starvation**	S6	562-569	-
5	1	Tissue specific metabolism- Metabolic profile of major organs- Brain, Muscle, Liver and Adipose tissue	S1 S4	893-897 763-780	-
6	1	Tissue specific metabolism- Metabolic profile of major organs-	S1	882-900	-
7	1	Metabolic disorders- Gout, SCID.	S2	375-380	
8	1	Revision and discussion of possible question	-	-	-
		Total No of Hours Planned For Unit V : 08			
1	1	Previous year End Semester Exam- QP discussion	-	-	-
2	1	Previous year End Semester Exam- QP discussion	-	-	-
Total	**02**	Hours planned for QP discussion : 03			
Total number of hours planned for this syllabi : 44					

SUGGESTED READINGS

S1: Lehninger, L., Nelson, D.L., and Cox, M.M., (2012). Principles of Biochemistry, 6th edition WH Freeman and Company, New York.

S2: Murray, R.K., Bender, D.A., Botham, K.M., and Kennelly, P.J., (2012). Harper's illustrated Biochemistry, 29th Edition. McGraw-Hill Medical. London.

S3: Donald Voet and Judith Voet , 2004. Biochemistry, John Wiley and Sons, 2nd Eedition. New York

S4: Leubert Stryer, 2009. Biochemistry, W.H. Freeman and Company. New York.

S5: Pamila C. Champ and Richard A. Harvey ,2008. Biochemistry, Lipponcott Company, Philadelphia.

S6: Smith. 2003. Principles of Biochemistry, McGraw– Hill International Book Company, London.

S7: Zubay, G., (2009). Biochemistry, W.C Brown Publishers, Saunders and Company, Philadelphia.

WEBSITES

W2 : http://www.omic online,org…/Classification/pathophysiology

W31: http://www.webmed.com/Heart disease

W32: https://www.ncbi.nih.gov/pubmed

UNIT-I

SYLLABUS

Introduction to control of enzyme activity: Allosteric interaction; Reversible covalent modification; proteolytic action; control of amount of enzyme; control of rates of enzyme degradation; feed back inhibition; feed forward stimulation. Role of compartmentation. Elucidation of Metabolic pathways- Single-and Multi-step pathways. Experimental approaches to study the metabolism- using metabolic inhibitors and isotopes.

INTRODUCTION TO CONTROL OF ENZYME ACTIVITY

A variety of mechanisms exist for controlling enzyme activity giving a full range from very fine to coarse control.

Regulatory molecules. Enzyme activity may be turned "up" or "down" by activator and inhibitor molecules that bind specifically to the enzyme.

Cofactors. Many enzymes are only active when bound to non-protein helper molecules known as cofactors.

Compartmentalization. Storing enzymes in specific compartments can keep them from doing damage or provide the right conditions for activity.

Feedback inhibition. Key metabolic enzymes are often inhibited by the end product of the pathway they control (feedback inhibition).

1.REGULATORY MOLECULES

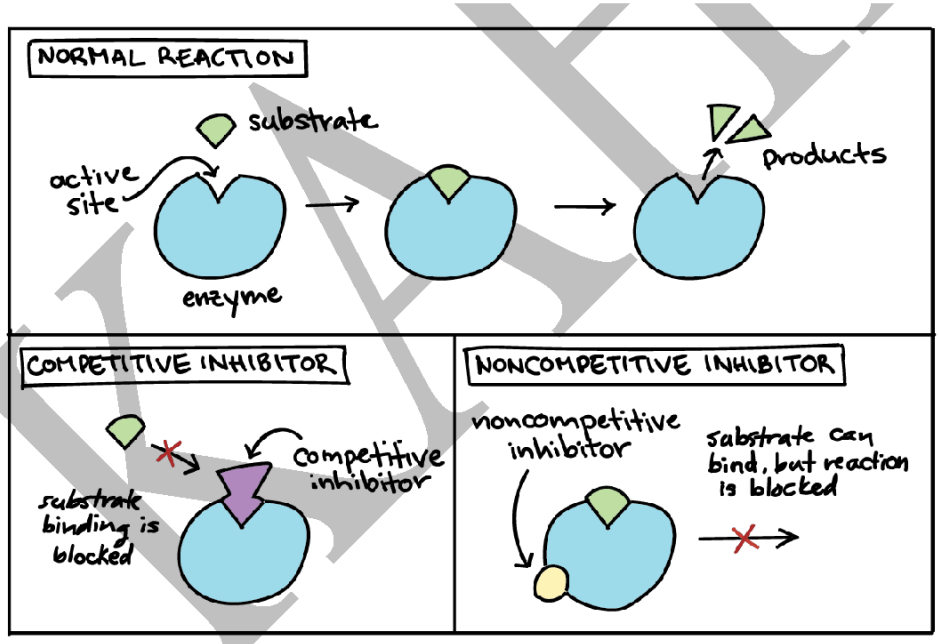
- Enzymes can be regulated by other molecules that either increase or reduce their activity. Molecules that increase the activity of an enzymes are called **activators**, while molecule that decrease activity of an enzyme are called **inhibitors**.
- There are many kinds of molecules that block or promote enzyme function, and that affect enzyme function by different routes.
- **Competitive vs. noncompetitive**
- In many well-studied cases, an activator or inhibitor's binding is reversible, meaning that the molecule doesn't permanently attach to the enzyme. Some important types of drugs

act as reversible inhibitors. For example, the drug tipranivir, blocks activity of a viral enzyme that helps the virus make more copies of itself.

Reversible inhibitors are divided into groups based on their binding behavior divided into two important groups: competitive and noncompetitive inhibitors.

An inhibitor may bind to an enzyme and block binding of the substrate, for example, by attaching to the active site. This is called **competitive inhibition**, because the inhibitor "competes" with the substrate for the enzyme. That is, only the inhibitor or the substrate can be bound at a given moment.

In **noncompetitive inhibition**, the inhibitor doesn't block the substrate from binding to the active site. Instead, it attaches at another site and blocks the enzyme from doing its job. This inhibition is said to be "noncompetitive" because the inhibitor and substrate can both be bound at the same time.

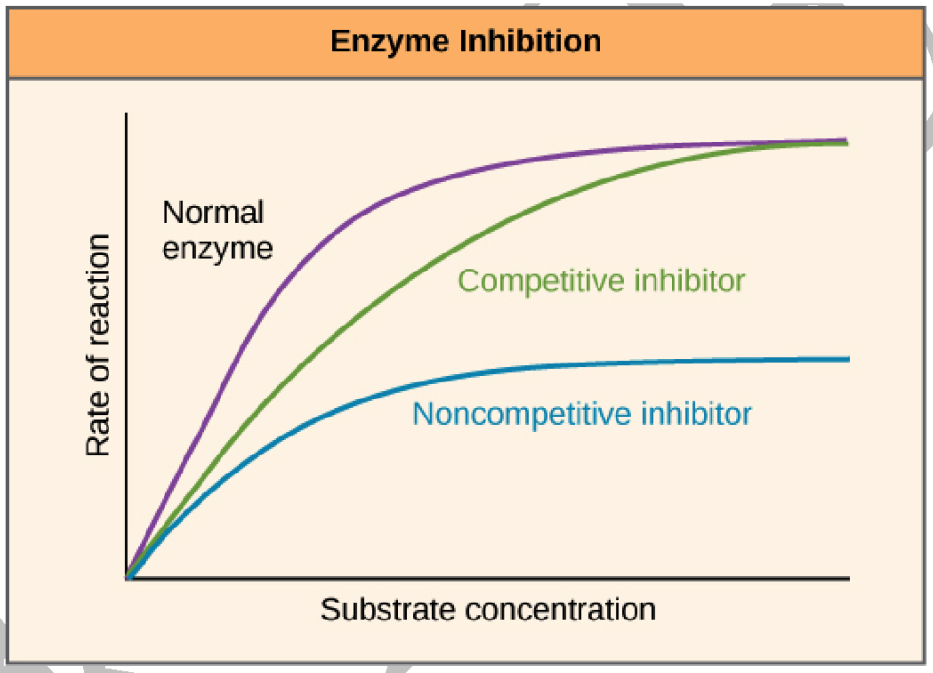


Competitive and non-competitive inhibitors can be told apart by how they affect an enzyme's activity at different substrate concentrations.

- If an inhibitor is competitive, it will decrease reaction rate when there's not much substrate, but can be "out-competed" by lots of substrate. That is, the enzyme can still reach its

maximum reaction rate given enough substrate. In that case, almost all the active sites of almost all the enzyme molecules will be occupied by the substrate rather than the inhibitor.

- If an inhibitor is noncompetitive, the enzyme-catalyzed reaction will never reach its normal maximum rate even with a lot of substrate. This is because the enzyme molecules with the noncompetitive inhibitor bound are "poisoned" and can't do their job, regardless of how much substrate is available.
- On a graph of reaction velocity (y-axis) at different substrate concentrations (x-axis), we can tell these two types of inhibitors apart by the shape of the curves:



M i x e d

A **mixed inhibitor** is a molecule that binds to an allosteric site on the enzyme, causing a conformational change that decreases catalytic activity at the active site. Mixed inhibitors generally have a preference towards binding either the enzyme-substrate complex or the enzyme alone.

U n c o m p e t i t i v e

An **uncompetitive inhibitor** is a molecule that binds only to the enzyme-substrate complex, rendering it catalytically inactive.

Inhibition type	Binding state	Binding site	Blocks substrate	Effect on K_m	Effect on V_{max}	Overcome by ↑[S]
Competitive	E	active site	yes	increases	no effect	yes
Noncompetitive	E or ES	allosteric site	no	no effect	decrease	no
Mixed	E or ES	allosteric site	no	increases or decreases	decrease	partially
Uncompetitive	ES	allosteric site	no	decreases	decrease	no

In living cells, there are hundreds of different enzymes working together in a coordinated manner. Living cells neither synthesize nor breakdown more material than is required for normal metabolism and growth. All of this necessitates precise control mechanisms for turning metabolic reactions on and off. Enzymes can be controlled or regulated in two ways: controlling the synthesis of the enzyme (genetic control) and controlling the activity of the enzyme (feedback inhibition).

2. GENETIC CONTROL

Genetic control of enzyme activity refers to controlling transcription of the mRNA needed for an enzyme's synthesis. In prokaryotic cells, this involves the induction or repression of enzyme synthesis by regulatory proteins that can bind to DNA and either block or enhance the function of RNA polymerase, the enzyme required for transcription. The regulatory proteins are part of either an operon or a regulon. An operon is a set of genes transcribed as a polycistronic message that is collectively controlled by a regulatory protein. A regulon is a set of related genes controlled by the same regulatory protein but transcribed as monocistronic units. Regulatory proteins may function either as **repressors** or **activators**.

Repressors

Repressors are regulatory proteins that block transcription of mRNA. They do this by binding to a portion of DNA called the operator that lies downstream of a promoter. The binding of the regulatory protein to the operator prevents RNA polymerase from passing the operator and transcribing the coding sequence for the enzymes. This is called negative control. Repressors are allosteric proteins that have a binding site for a specific molecule. Binding of that molecule to the allosteric site of the repressor can alter the repressor's shape that, in turn affects its ability to bind to DNA. This can work in one of two ways:

Some repressors are synthesized in a form that cannot by itself bind to the operator. The binding of a molecule called a corepressor, however, alters the shape of the regulatory protein to a form that can bind to the operator and block transcription.

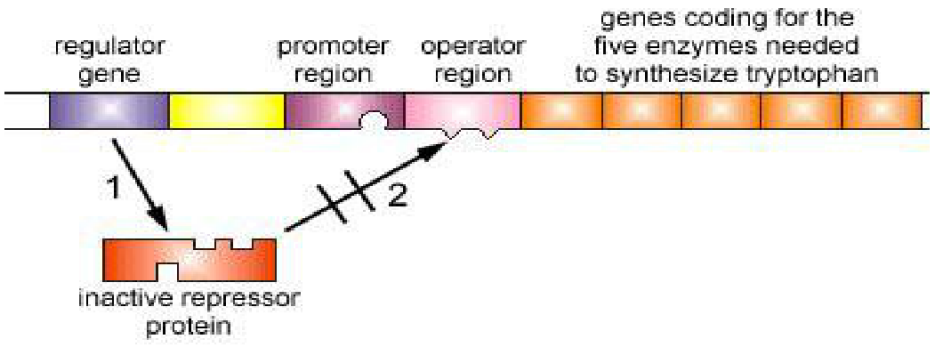


Figure : A Repressible Operon in the Absence of a Corepressor (The Tryptophan Operon). Step 1: The regulator gene codes for an inactive repressor protein. Step 2: The inactivated repressor protein is unable to bind to the operator region of the operon.

An example of this type of repression is the trp operon in E. coli that encodes the five enzymes in the pathway for the biosynthesis of the amino acid tryptophan. In this case, the repressor protein, coded for by a regulatory gene, normally does not bind to the operator region of the trp operon and the five enzymes needed to synthesize the amino acid tryptophan are made.

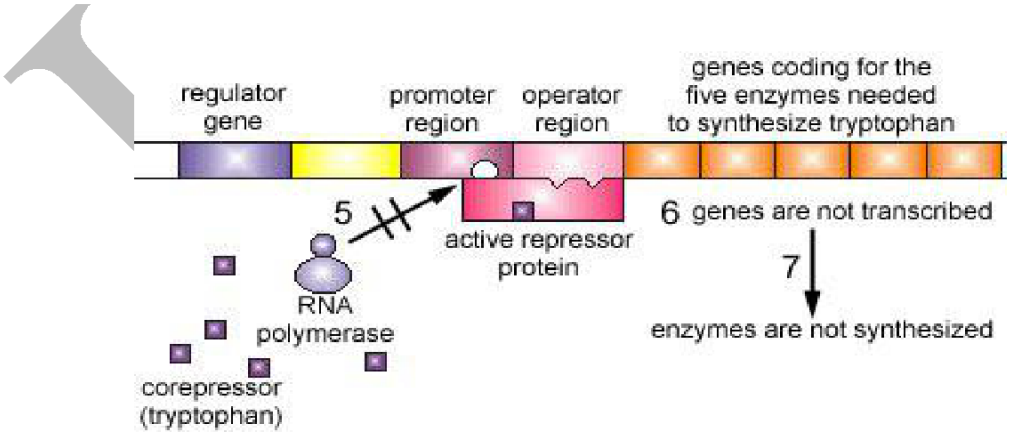


Figure : A Repressible Operon in the Presence of a Corepressor (The Tryptophan Operon). Step 5: With the active repressor protein bound to the operator region, RNA polymerase (the enzyme responsible for the transcription of genes) is unable to bind to the promoter region of the operon.

Step 6: If RNA polymerase does not bind to the promoter region, the five enzyme genes are not transcribed into mRNA. Step 5: Without the transcription of the five genes, the five enzymes needed for the bacterium to synthesize the amino acid tryptophan are not made.

Induction

However, the binding of a molecule called an inducer alters the shape of the regulatory protein in a way that now blocks its binding to the operator and thus permits transcription. An example of this is the lac operon that encodes for the three enzymes needed for the degradation of lactose by E. coli. E. coli will only synthesize the three enzymes it requires to utilize lactose if that sugar is present in the surrounding environment. In this case, lactose functions as an inducer. In the absence of lactose, the repressor protein binds to the operatorand RNA polymerase is unable to get beyond the operator and transcribe the genes for utilization of lactose and the three enzymes for degradation of lactose are not synthesized.

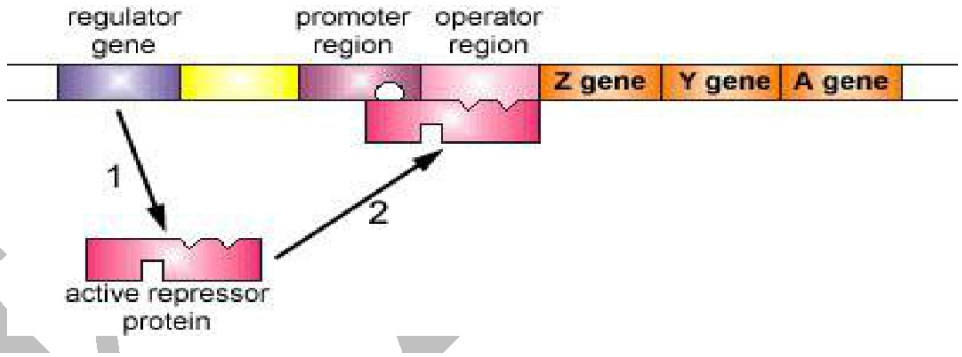


Figure : An Inducible Operon in the Absence of an Inducer (The Lactose Operon). Step 1: Theregulator gene codes for an active repressor protein.

Step 2: The repressor protein then binds to the operator region of the operon.

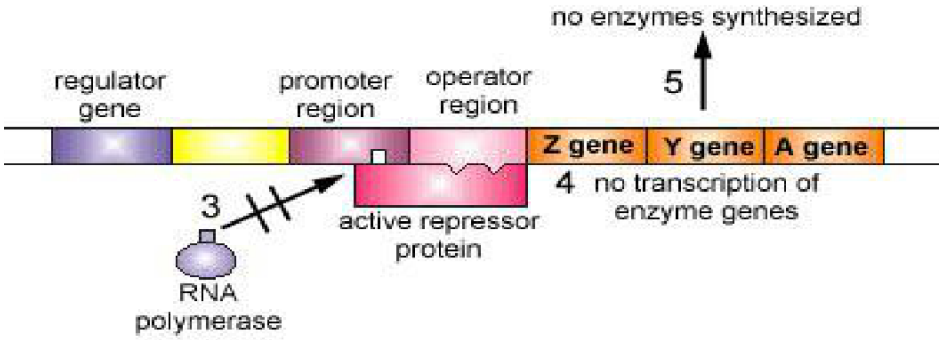


Figure : An Inducible Operon in the Absence of an Inducer (The Lactose Operon). Step 3: With the active repressor protein bound to the operator region, RNA polymerase (the enzyme responsible for the transcription of genes) is unable to bind to the promoter region of the operon. Step 4: If RNA polymerase does not bind to the promoter region, the three enzyme genes (Z, Y, and A) are not transcribed into mRNA. Step 5: Without the transcription of the three enzyme genes, the three enzymes needed for the utilization of the sugar lactose by the bacterium are not synthesized.

When lactose, the inducer, is present, it binds to the allosteric repressor protein and causes it to change shape in such a way that it is no longer able to bind to the operator. Now RNA polymerase can transcribe the three genes required for the degradation of lactose and the bacterium is able to synthesize the enzymes needed for its utilization.

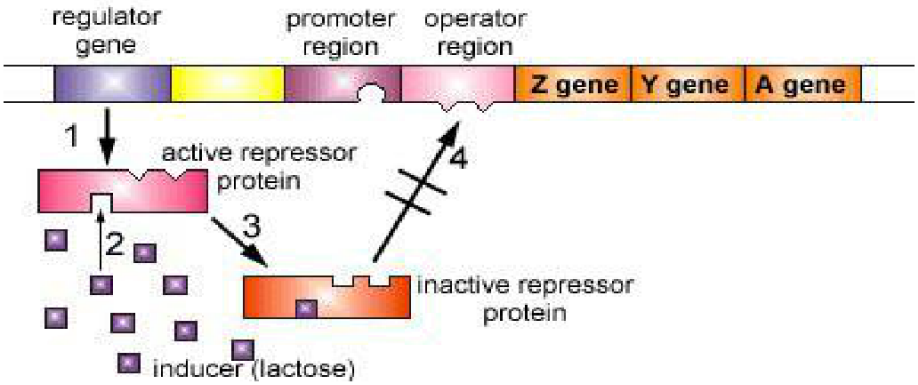


Fig. : An Inducible Operon in the Presence of an Inducer (The Lactose Operon)Step 1: The regulator gene codes for an active repressor protein. Step 2: Lactose, the inducer molecule binds to the active repressor protein. Step 3: The binding of the inducer inactivates the repressor protein. Step 4: The inactivated repressor protein is then unable to bind to the operator region of the operon.

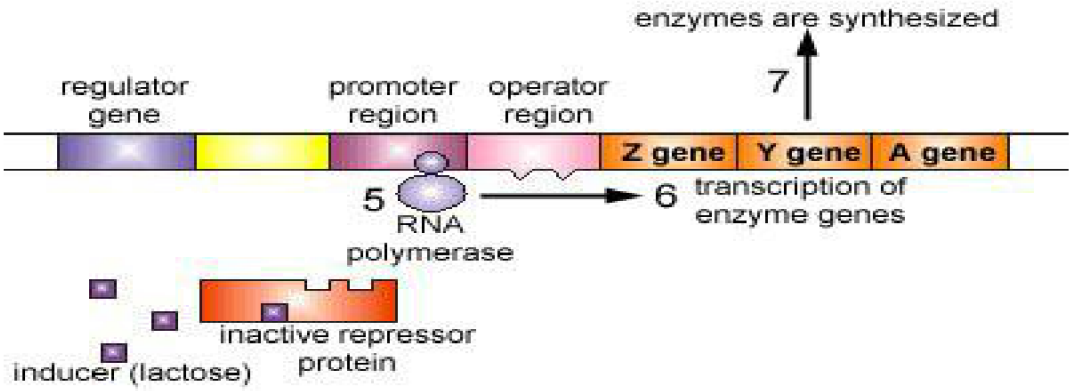


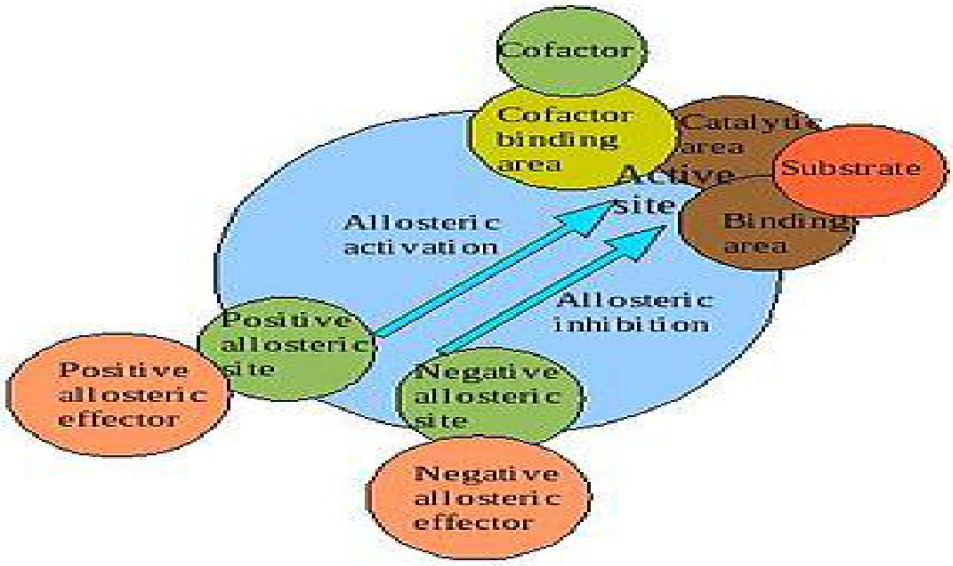
Fig. : An Inducible Operon in the Presence of an Inducer (The Lactose Operon)Step 5: Since the inactive repressor protein is unable to bind to the operator region, RNA polymerase (the enzyme responsible for the transcription of genes) is now able to bind to the promoter region of the operon. Step 6: RNA polymerase is now able to transcribe the three enzyme genes (Z, Y, and A) into mRNA. Step 7: With the transcription of these genes, the three enzymes needed for the bacterium to utilize the sugar lactose are now synthesized. (The Z gene codes for beta-galactosidase, an enzyme that breaks down lactose into glucose and galactose. The Y gene codes for permease, an enzyme which transports lactose into the bacterium. The A gene codes for transacetylase, an enzyme which is thought to aid in the release of galactosides.)

3.ALLOSTERIC ENZYMES

This type of enzymes presents two binding sites: the substrate of the enzyme and the effectors. Effectors are small molecules which modulate the enzyme activity; they function through reversible, non-covalent binding of a regulatory metabolite in the allosteric site (which is not the active site). When bound, these metabolites do not participate in catalysis directly, but they are still essential: they lead to conformational changes in a concrete part of the enzyme.

These changes affect the overall conformation of the active site, causing modifications on the activity of the reaction.

In biochemistry, **allosteric regulation** is the regulation of an enzyme or other protein by binding an effector molecule at the protein's allosteric site (that is, a site other than the protein's active site). Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors. The term allostery comes from the Greek allos, "other", and stereos, "solid (object)", in reference to the fact that the regulatory site of an allosteric protein is physically distinct from its active site. Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feedforward from upstream substrates. Long-range allostery is especially important in cell signaling.



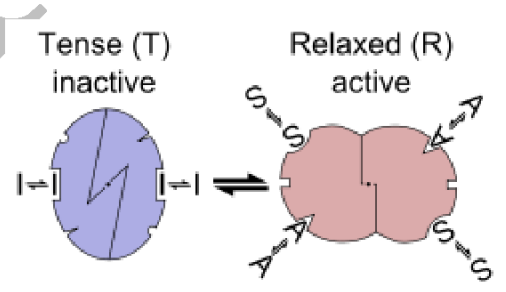


Fig: Allosteric transition of an enzyme between R and T states, stabilized by an agonist, an inhibitor and a substrate (the MWC model)

Allosteric sites are sites on the enzyme that bind to molecules in the cellular environment. The sites form weak, noncovalent bonds with these molecules, causing a change in the conformation of the enzyme. This change in conformation translates to the active site, which then affects the reaction rate of the enzyme. Allosteric interactions can both inhibit and activate enzymes and are a common way that enzymes are controlled in the body

Properties

Allosteric enzymes are generally larger in mass than other enzymes. Different from having a single subunit enzyme, in this case they are composed of multiple subunits, which contain active sites and regulatory molecule binding sites.

They present a special kinetics: the cooperation. In here, configuration changes in each chain of the protein strengthen changes in the other chains. These changes occur at the tertiary and quaternary levels of organisation.

Based on modulation, they can be classified in two different groups:

- Homotropic allosteric enzymes:.
- Heterotropic allosteric enzymes:

Types of allosteric regulation

Homotropic

A homotropic allosteric modulator is a substrate for its target enzyme, as well as a regulatory molecule of the enzyme's activity. It is typically an activator of the enzyme.

Heterotropic

A heterotropic allosteric modulator is a regulatory molecule that is not also the enzyme's substrate. It may be either an activator or an inhibitor of the enzyme. Some allosteric proteins can be regulated by both their substrates and other molecules. Such proteins are capable of both homotropic and heterotropic interactions.

Non-Regulatory Allostery

A non-regulatory allosteric site refers to any non-regulatory component of an enzyme (or any protein) that is not itself an amino acid. For instance, many enzymes require sodium binding

to ensure proper function. However, the sodium does not necessarily act as a regulatory subunit; the sodium is always present and there are no known biological processes to add/remove sodium to regulate enzyme activity. Non-regulatory allostery could comprise any other ions besides sodium (calcium, magnesium, zinc), as well as other chemicals and possibly vitamins.

Positive modulation

Positive allosteric modulation (also known as allosteric activation) occurs when the binding of one ligand enhances the attraction between substrate molecules and other binding sites. An example is the binding of oxygen molecules to hemoglobin, where oxygen is effectively both the substrate and the effector. The allosteric, or "other", site is the active site of an adjoining protein subunit. The binding of oxygen to one subunit induces a conformational change in that subunit that interacts with the remaining active sites to enhance their oxygen affinity.

Negative modulation

Negative allosteric modulation (also known as allosteric inhibition) occurs when the binding of one ligand decreases the affinity for substrate at other active sites. For example, when 2,3-BPG binds to an allosteric site on hemoglobin, the affinity for oxygen of all subunits decreases. This due to when a regulator is absent from the binding site.

4. COVALENT MODIFICATION

, the active and inactive form of the enzymes are altered due to covalent modification of their structures which is catalysed by other enzymes. This type of regulation consists of the addition or elimination of some molecules which can be attached to the enzyme protein. The most important groups that work as modifiers are **phosphate, methyl, uridine, adenine and adenosine diphosphate ribosyl**. These groups are joined to or eliminated from the protein by other enzymes. The most remarkable covalent modification is phosphorylation. Serine, Tryptophan and Tyrosine are common amino acids that participate in covalent modifications and are used to control enzyme's catalytic activities. Kinase and phosphatases are commonly known enzymes that affect these modifications, which result in shifting of conformational states of the binding affinity to substrate.

Phosphorylation

Phosphorylation is the addition of phosphate groups to proteins, which is the most frequent regulatory modification mechanism in our cells. This process takes place in prokaryotic and eukaryotic cells (in this type of cells, a third or a half of the proteins experience phosphorylation). Because of its frequency, phosphorylation has a lot of importance in regulatory pathways in cells.

The addition of a phosphoryl group to an enzyme is catalysed by kinase enzymes, while the elimination of this group is catalysed by phosphatase enzymes. The frequency of phosphorylation as a regulatory mechanism is due to the ease of changing from phosphorylated form to dephosphorylated form.

Phosphorylation or dephosphorylation make the enzyme be functional at the time when the cell needs the reaction to happen. The effects produced by the addition of phosphoryl groups that regulate the kinetics of a reaction can be divided in two groups:

- Phosphorylation changes the conformation of an enzyme to a more active or inactive way (e.g. regulation of glycogen phosphorylase). Each phosphate group contains two negative charges, so the addition of this group can cause an important change in the conformation of the enzyme. The phosphate can attract positively charged amino acids or create repulsive interactions with negatively charged amino acids. These interactions can change the conformation and the function of the enzyme. When a phosphatase enzyme removes the phosphate groups, this enzyme returns to its initial conformation.
- Phosphorylation modifies the affinity of the enzyme to the substrate (e.g. phosphorylation of isocitrate dehydrogenase creates electrostatic repulsion which inhibits the union of the substrate to the active center). Phosphorylation can take place in the active center of the enzyme. It can change the conformation of this active center, so it can recognize the substrate or not. Also, the ionized phosphate can attract some parts of the substrate, which can join to the enzyme.

Phosphorylation and dephosphorylation may take place as a result of the response to signals that warn about a change in the cell state. This means that some pathways where regulatory enzymes participate are regulated by phosphorylation after a specific signal: a change in the cell.

Some enzymes can be phosphorylated in multiple sites. The presence of a phosphoryl group in a part of a protein may depend on the folding of the enzyme (which can make the protein more or less accessible to kinase proteins) and the proximity of other phosphoryl groups.

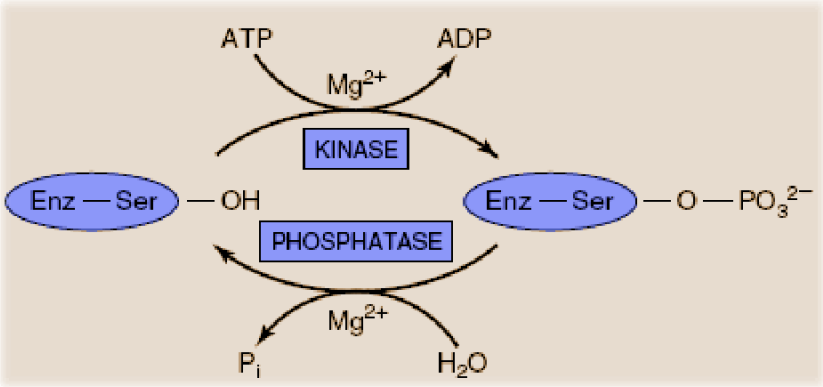


Figure: Covalent modification of a regulated enzyme by phosphorylation-dephosphorylation of a seryl residue.

The ease of interconversion of enzymes between their phospho- and dephospho- forms in part accounts for the frequency of phosphorylation-dephosphorylation as a mechanism for regulatory control. Phosphorylation-dephosphorylation permits the functional properties of the affected enzyme to be altered only for as long as it serves a specific need. Once the need has passed, the enzyme can be converted back to its original form, poised to respond to the next stimulatory event. A second factor underlying the widespread use of protein phosphorylation-dephosphorylation lies in the chemical properties of the phosphoryl group itself. In order to alter an enzyme's functional properties, any modification of its chemical structure must influence the protein's three-dimensional configuration.

5. Control of enzyme activity by Proteolytic action

Proteolysis

Enzymes need to go through a maturation process to be activated. A precursor (inactive state, better known as zymogen) is first synthesized, and then, by cutting some specific peptide bonds (enzymatic catalysis by hydrolytic selective split), its 3D conformation is highly modified into a catalytic functional status, obtaining the active enzyme.

Proteolysis is irreversible and normally a non-specific process. The same activator can modulate different regulatory enzymes : once trypsin is activated, it activates many other hydrolytic enzymes. Proteolysis can also be fast and simple so the hydrolysis of a single peptide bond can be enough to change the conformation of the protein and build an active zone, allowing the interaction between the enzyme and the substrate, for instance, chymotrypsin activation (as it can be seen in the images).

Many different types of proteins with different roles in metabolism are activated by proteolysis for big reasons:

- Powerful hydrolytic enzymes, for instance, digestive enzymes, are activated by proteolysis so we can ensure that they are unable to hydrolyze any unwilling protein until they get to the right place: hydrolyzing protein zymogens are synthesized at the pancreas and accumulated in vesicles where they remain harmless. When they are needed, some hormonal or nervous stimulus triggers the release of the zymogens right to the intestine and they are activated.
- Some eventual responses must be immediate so enzymes that catalyze those reactions need to be prepared but not active, for that reason a zymogene is synthesized and stays ready for being rapidly activated. Coagulation response is based on enzymatic cascade proteolysis maturation. So, by activating one first catalyzing enzyme a big amount of the following enzymes is activated and the amount of product required is achieved as it is needed.
- Connective tissues proteins as collagen (zymogen: procolagen), hormones like insulin (zymogen: proinsulin) and proteins involved in development processes and apoptosis (programmed cell death) are activated by proteolysis too.

Proteolysis is irreversible, which implies the need of a process of enzyme deactivation. Specific inhibitors, analogous to the substrate, will strongly join the enzyme, blocking the substrate to join the enzyme. This union may last for months

7. COFACTORS AND COENZYMES

Cofactors

Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules called cofactors to be bound for activity. Cofactors can be

either inorganic (e.g., metal ions and iron-sulfur clusters) or organic compounds (e.g., flavin and heme). Organic cofactors can be either prosthetic groups, which are tightly bound to an enzyme, or coenzymes, which are released from the enzyme's active site during the reaction. Coenzymes include NADH, NADPH and adenosine triphosphate. These molecules transfer chemical groups between enzymes.

An example of an enzyme that contains a cofactor is carbonic anhydrase, and is shown in the ribbon diagram above with a zinc cofactor bound as part of its active site.[52] These tightly bound molecules are usually found in the active site and are involved in catalysis. For example, flavin and heme cofactors are often involved in redox reactions.

Enzymes that require a cofactor but do not have one bound are called apoenzymes or apoproteins. An apoenzyme together with its cofactor(s) is called a holoenzyme (this is the active form). Most cofactors are not covalently attached to an enzyme, but are very tightly bound. However, organic prosthetic groups can be covalently bound (e.g., thiamine pyrophosphate in the enzyme pyruvate dehydrogenase). The term "holoenzyme" can also be applied to enzymes that contain multiple protein subunits, such as the DNA polymerases; here the holoenzyme is the complete complex containing all the subunits needed for activity.

Coenzymes

Coenzymes are small organic molecules that can be loosely or tightly bound to an enzyme. Tightly bound coenzymes can be called allosteric groups. Coenzymes transport chemical groups from one enzyme to another. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins (compounds which cannot be synthesized by the body and must be acquired from the diet). The chemical groups carried include the hydride ion (H^-) carried by NAD or $NADP^+$, the phosphate group carried by adenosine triphosphate, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use the coenzyme NADH.

Coenzymes are usually continuously regenerated and their concentrations maintained at a steady level inside the cell: for example, NADPH is regenerated through the pentose phosphate pathway and S-adenosylmethionine by methionine adenosyltransferase. This continuous regeneration means that even small amounts of coenzymes are used very intensively. For example, the human body turns over its own weight in ATP each day.[

8. FEEDBACK INHIBITION

Feedback inhibition, in enzymology, suppression of the activity of an enzyme, participating in a sequence of reactions by which a substance is synthesized, by a product of that sequence. When the product accumulates in a cell beyond an optimal amount, its production is decreased by inhibition of an enzyme involved in its synthesis. After the product has been utilized or broken down and its concentration thus decreased, the inhibition is relaxed, and the formation of the product resumes. Such enzymes, whose ability to catalyze a reaction depends upon molecules other than their substrates (the ones upon which they act to form a product), are said to be under allosteric control. Feedback inhibition is a mechanism by which the concentration of certain cell constituents is limited.

An example of feedback inhibition in human cells is the protein aconitase (an enzyme that catalyses the isomeration of citrate to isocitrate). When the cell needs iron, this enzyme loses the iron molecule and its form changes. When this happens, the aconitase is converted to IRPF1, a translation repressor or mRNA stabilizer that represses the formation of iron-binding proteins and favours formation of proteins that can get iron from the cell's reservations

The entire process of **respiration** is also **regulated by feedback inhibition**. Recall that the process involves converting glucose to CO_2 and water and forming ATP in the process. Glucose is first converted to pyruvate or pyruvic acid, which then forms acetyl CoA and enters the citric acid (TCA) cycle.

The first step in the citric acid (TCA) cycle involves combining acetyl CoA with a molecule of oxaloacetate or oxaloacetic acid to form a molecule of citric acid or citrate. The enzyme which catalyzes this step, citrate synthase, is inhibited by ATP. When the cell has a low concentration of ATP, in other words, is low in energy, the citric acid (TCA) cycle operates, feeds the electron transport chain, and ATP is produced. As the energy store of the cell builds up

and the concentration of ATP increases, the TCA cycle or citric acid cycle shuts down because the ATP inhibits the citrate synthase and stops the formation of citrate (or citric acid) which is needed for the cycle to operate.

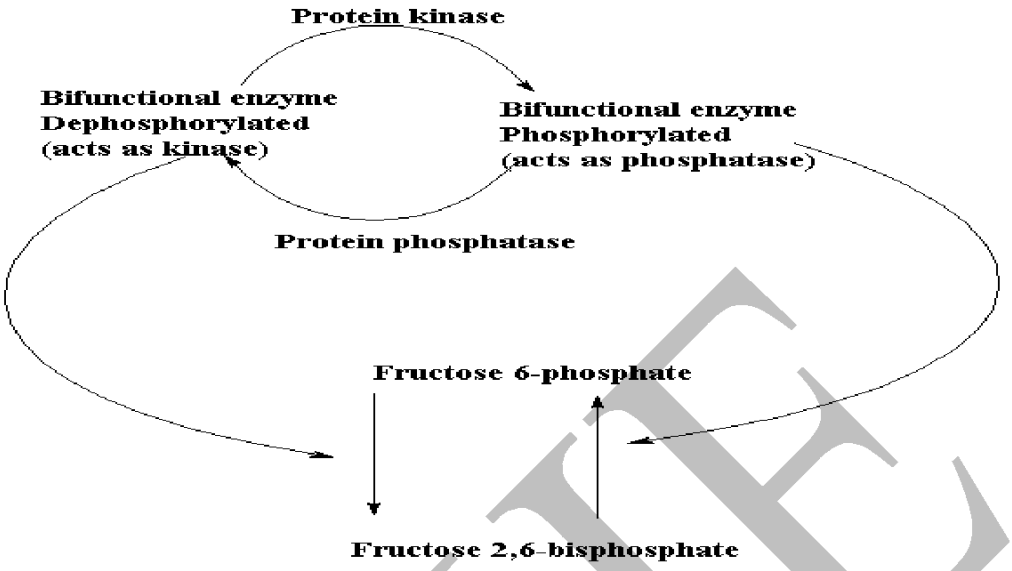
9. FEED-FORWARD STIMULATION

Feed-forward is a term describing an element or pathway within a control system which passes a controlling signal from a source in the control system's external environment, often a command signal from an external operator, to a load elsewhere in its external environment. A control system which has only feed-forward behavior responds to its control signal in a pre-defined way without responding to how the load reacts; it is in contrast with a system that also has feedback, which adjusts the output to take account of how it affects the load, and how the load itself may vary unpredictably; the load is considered to belong to the external environment of the system.

Some prerequisites are needed for control scheme to be reliable by pure feed-forward without feedback: the external command or controlling signal must be available, and the effect of the output of the system on the load should be known (that usually means that the load must be predictably unchanging with time). Sometimes pure feed-forward control without feedback is called 'ballistic', because once a control signal has been sent, it cannot be further adjusted; any corrective adjustment must be by way of a new control signal. In contrast 'cruise control' adjusts the output in response to the load that it encounters, by a feedback mechanism.

Eg-Relationship between glucose levels and [fructose 2,6-bisphosphate]

PFK2 and FBase2 are reciprocally regulated by phosphorylation on a serine residue. This reaction is carried out by a protein kinase that is activated by cAMP in response to low blood glucose levels. The figure below illustrates the effect of glucose levels on these pathways. To see which pathways are activated when blood glucose levels are high or low position the mouse over the appropriate button below.



High glucose levels- Glycolysis is stimulated

Low glucose levels - Glycolysis is inhibited

To summarize the effects of blood glucose levels.

- **High blood sugar**
  - Increase in [Glucose] ⟶ Increase in [Glucose 6-phosphate] ⟶ Increase in [Fructose 6-phosphate] ⟶ Increase in [Fructose 2,6-bisphosphate]
  - Stimulation of phosphofructokinase--Feed forward stimulation
- **Low blood sugar**
  - Decrease in [Glucose] ⟶ Increase in [Glucagon] ⟶ Phosphorylation of bifunctional enzyme (by cAMP activated protein kinase) ⟶ Stimulate FBase2, Inhibit PFK2 ⟶ Decreases [Fructose 2,6-bisphosphate] ⟶ ATP can inhibit glycolysis
  - Glucose preserved for use by organs such as brain

10. ROLE OF COMPARTMENTATION

Two of the three processes of respiration, the TCA cycle and oxidative phosphorylation, occur within the mitochondrion, whilst the third, glycolysis, occurs in the cytoplasm and supplies

the mitochondria with substrates such as pyruvate from the oxidation of carbohydrate. Protein and lipid degradation can also provide substrates, feeding into the TCA cycle as pyruvate, 2oxoglutarate and oxaloacetate, from amino acid metabolism, and as acetyl CoA following β oxidation. As well as providing reductant (NADH, $FADH_2$) for generation of ATP, the TCA cycle serves as an important source of carbon skeletons for biosynthetic processes.The biosynthetic function, whilst not unique to plants, is likely to be more important in the mitochondria of an autotrophic organism. Even so, the major demand on the mitochondria within photosynthetic cells is for the oxidation of glycine that is generated during photorespiration. As photorespiration originates in the chloroplast, due to the oxygenase reaction of Rubisco, glycine oxidation is intrinsically linked with photosynthesis.

There are thus a number of different requirements for mitochondrial metabolism, ranging from biosynthesis to catabolic processes that may or may not result in ATP synthesis. Although it is feasible for all of these processes to occur simultaneously within the same organelle, it is equally likely that, as the metabolic demands differ between cells and tissues, so does the composition and activity of the mitochondria. There is increasing evidence that mitochondria do indeed differ in protein composition in different types of plant tissue.

ELUCIDATION OF METABOLIC PATHWAYS

In biochemistry, **metabolic pathways** are series of chemical reactions occurring within a cell. In each pathway, a principal chemical is modified by a series of chemical reactions. Enzymes catalyze these reactions, and often require dietary minerals, vitamins, and other cofactors in order to function properly. Because of the many chemicals (a.k.a. "metabolites") that may be involved, metabolic pathways can be quite elaborate. In addition, numerous distinct pathways co-exist within a cell. This collection of pathways is called the metabolic network. Pathways are important to the maintenance of homeostasis within an organism. Catabolic (break-down) and Anabolic (synthesis) pathways often work interdependently to create new biomolecules as the final end-products.

A metabolic pathway involves the step-by-step modification of an initial molecule to form another product. The resulting product can be used in one of three ways:

- To be used immediately, as the end-product of a metabolic pathway

- To initiate another metabolic pathway, called a flux generating step
- To be stored by the cell

Each metabolic pathway consists of a series of biochemical reactions that are connected by their intermediates: the products of one reaction are the substrates for subsequent reactions, and so on. Metabolic pathways are often considered to flow in one direction. Although all chemical reactions are technically reversible, conditions in the cell are often such that it is thermodynamically more favorable for flux to flow in one direction of a reaction. For example, one pathway may be responsible for the synthesis of a particular amino acid, but the breakdown of that amino acid may occur via a separate and distinct pathway. One example of an exception to this "rule" is the metabolism of glucose. Glycolysis results in the breakdown of glucose, but several reactions in the glycolysis pathway are reversible and participate in the re-synthesis of glucose (gluconeogenesis).

- Glycolysis was the first metabolic pathway discovered:

1. As glucose enters a cell, it is immediately phosphorylated by ATP to glucose 6-phosphate in the irreversible first step.
2. In times of excess lipid or protein energy sources, certain reactions in the glycolysis pathway may run in reverse in order to produce glucose 6-phosphate which is then used for storage as glycogen or starch.

- Metabolic pathways are often regulated by feedback inhibition.
- Some metabolic pathways flow in a 'cycle' wherein each component of the cycle is a substrate for the subsequent reaction in the cycle, such as in the Krebs Cycle (see below).
- Anabolic and catabolic pathways in eukaryotes often occur independently of each other, separated either physically by compartmentalization within organelles or separated biochemically by the requirement of different enzymes and co-factors.

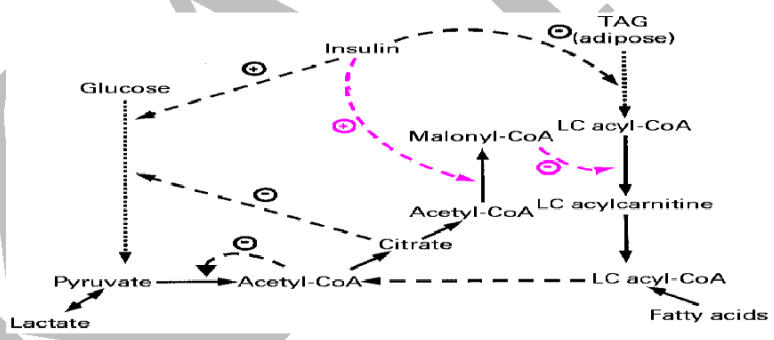
Metabolic Pathways - single and multi step

Metabolic pathways are composed of many single-step chemical reactions which release energy in manageable amounts. All living organisms must have some original source of energy, which is generally an organic molecule, or group of organic molecules which must be disassembled or digested prior to entry into the site where such pathways take place. Some

organisms, such as many bacteria and fungi begin the process of digestion outside of the body via the release of **exoenzymes**, which break large molecules down into forms which can cross the semi-permiable boundary of the plasma membrane. Some single-celled organisms engulf whole particles via phagocytosis or pinocytosis, thus they perform the process of digestion intracellularly, while large, multicellular organisms have evolved complex organ systems which perform the process of digestion prior to passage of nutrient molecules into the cell. Once digestion has occurred, nutrients can enter into those areas of the cell where metabolic pathways take place.

One of the most common forms of nutrients which can enter into the metabolic process is the monosaccharide glucose, which has the chemical formula $C_6H_{12}O_6$. This molecule is readily broken down by cells, and its elements utilized in various cellular activities. By degrading the glucose molecule via a series of enzyme-mediated reactions, the cell can make the most efficient use of the energy stored between the chemical bonds of it component parts. The process cells utilize in the breakdown of glucose is called **glycolysis**. which is a multistep pathway

Eg: Multi step pathway



Single step pathway

Example: Conversion of phenyl alanine to tyrosine whigh is stimulated byphenyl alanine hydroxylase

EXPERIMENTAL APPROACHES TO THE STUDY OF METABOLISM

Following points should be considered while studying metabolism

- Understanding metabolic pathways
- Sequence of reactions

- Mechanism of reaction
- Control or regulatory mechanism

Experimental Approaches to the Study of Metabolism

Use of metabolic inhibitors - build up of intermediates prior to the inhibited reaction

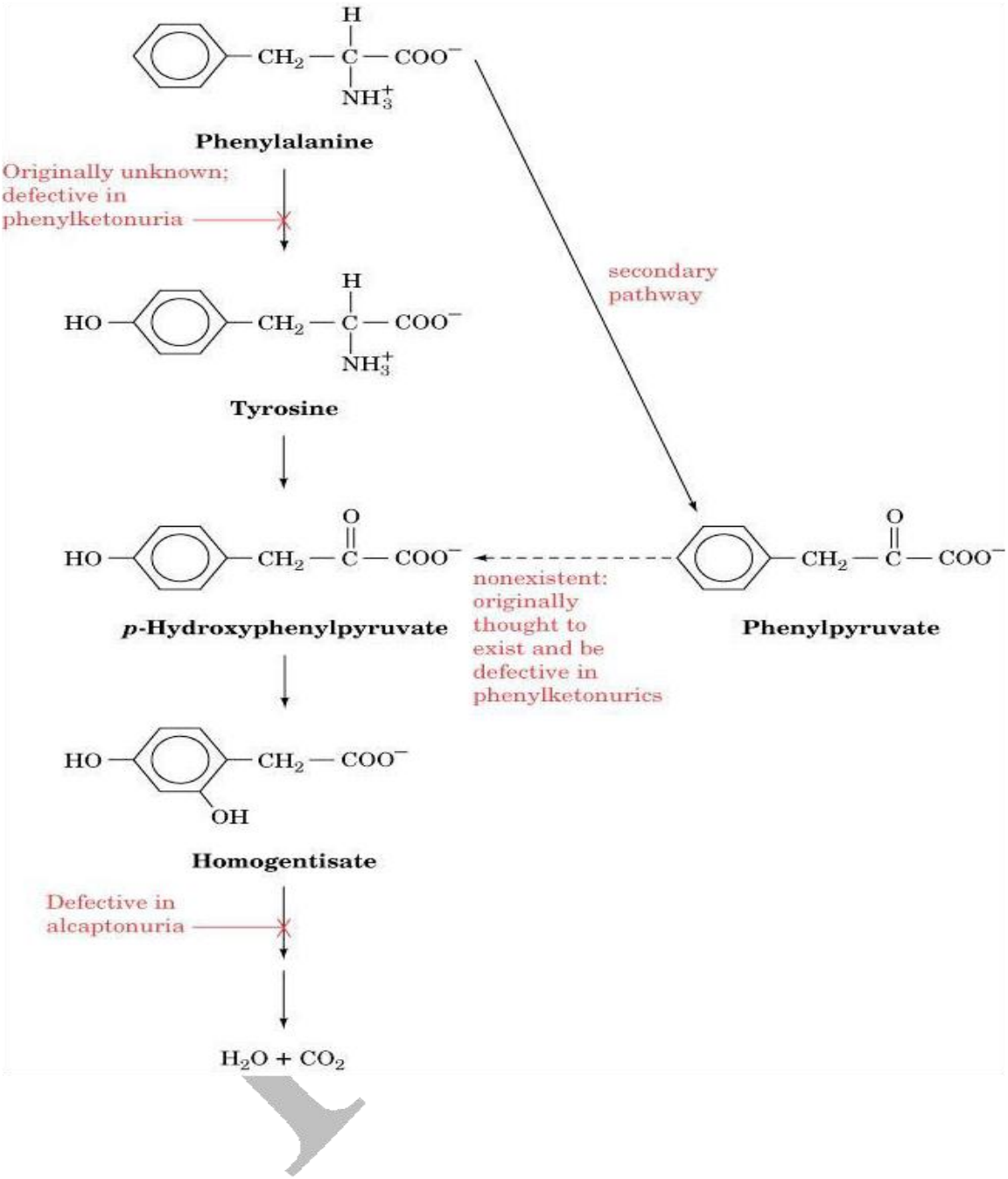
Use of growth studies - supply exogenous intermediate in the presence or absence of inhibitor, in genetically altered (natural or engineered) organism

Use of biochemical genetics - study naturally occurring organisms for defective processing of substances, manipulate organisms for absence or return of function

Use of Isotopes - atoms with same number of protons but different number of neutrons, same chemical properties

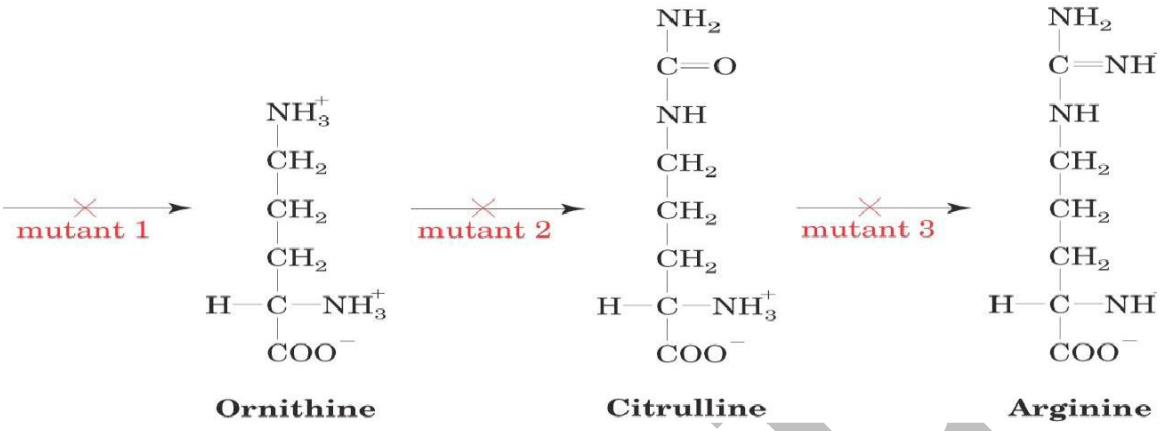
Use of Isolated organs, cells, and subcellular organelles

Eg:Pathway for phenylalanine degradation



Eg: Pathway of arginine biosynthesis indicating the positions of genetic blocks

Neurospora crassa auxotrophic mutantsin arginine biosynthesis



ISOTOPES IN BIOCHEMISTRY

- Isotopes, atoms with different number of neutrons
- used to label molecules without changing their chemical properties
- used for in vivo NMR studies, 1H, 13C, 31P
- radioactive isotopes (unstable), 3H, 14C, 32P, 35S
- alpha emitter (He)
- beta (electrons), 3H, 14C, 32P; 0.0018. 0.155, 1.71 MeV
- gamma (photons)
- detection by
- proportional counting (Geiger, gas charge)
- liquid scintillation counting (fluorescence)
- autoradiography (film)
- half-lives
- study precursor-product relation

Some Trace Isotopes of Biochemical Importance

Stable Isotopes

Nucleus	Natural Abundance (%)
^{2}H	0.015
^{13}C	1.07
^{15}N	0.37
^{18}O	0.20

Source: Holden, N.E., *in* Lide, D.R. (Ed.), *Handbook of Chemistry and Physics* (82nd ed.), *pp.* 11–51 to 197, CRC Press (2001).

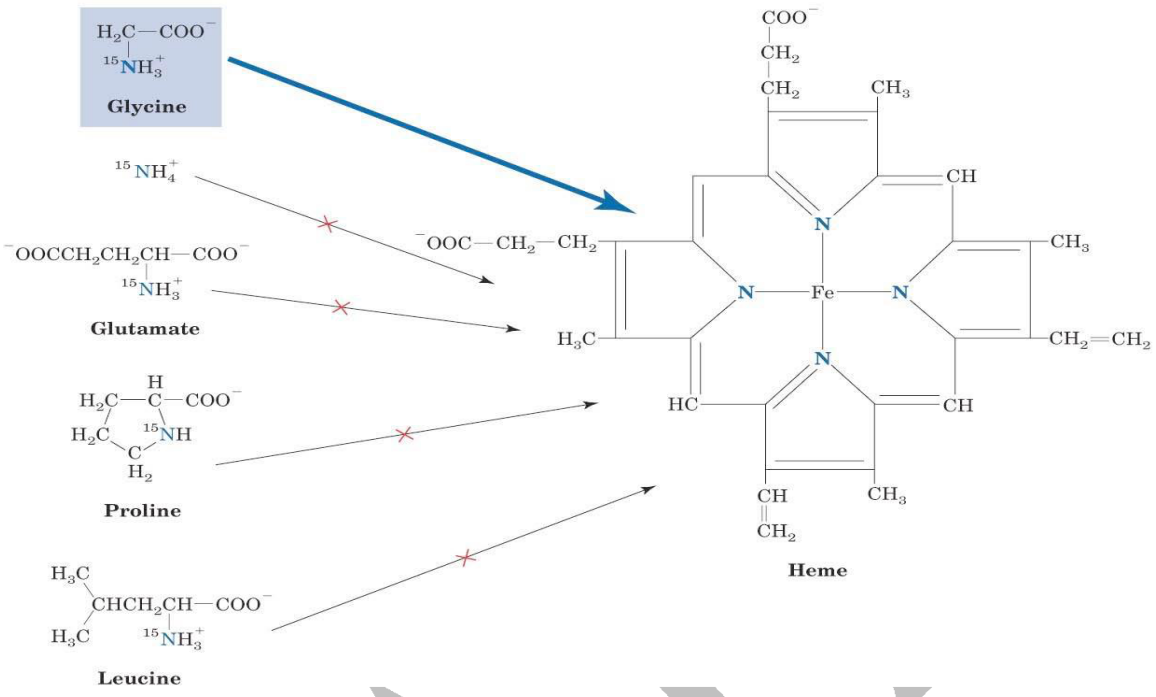
Some Trace Isotopes of Biochemical Importance

Radioactive Isotopes

Nucleus	Radiation Type	Half-Life
^{3}H	β	12.33 years
^{14}C	β	5715 years
^{22}Na	β^{+}, γ	2.60 years
^{32}P	β	14.28 days
^{35}S	β	87.2 days
^{45}Ca	β	162.7 days
^{60}Co	β, γ	5.271 years
^{125}I	γ	59.4 days
^{131}I	β, γ	8.04 days

Source: Holden, N.E., *in* Lide, D.R. (Ed.), *Handbook of Chemistry and Physics* (82nd ed.), *pp.* 11–51 to 197, CRC Press (2001).

The metabolic origin of the nitrogen atoms in heme



POSSIBLE QUESTIONS

2 mark questions

1. What is feed forward stimulation
2. Write short notes on compartmentation
3. Draw the structure of lac operon
4. Explain allosteric modification with an example
5. What is the role of compartmentation in regulation?
6. Explain enzyme induction with an example
7. What is allosteric enzyme? List the characters of this enzyme
8. Narrate the covalent modification with an example.
9. What is feed forward stimulation? Explain with an example
10. What is complementation group?
11. Narrate the feed back inhibition with an example.
12. List some of the inhibitors used in metabolic study
13. Give the role of isotopes in study of metabolism

Essay type questions (6 Marks)

1. How is the activity of enzyme modified by allosteric modification?
2. Elabrate the role of covalent modification in the control of enzyme activity
3. How will you elucidate the metabolic pathways
4. Explain the Feed back inhibition-types with an example
5. With an example mention the role of feed forward stimulation
6. Use of radioisotope in the elucidation of metabolic pathways
7. Explain enzyme induction and repression with neat diagram
8. Give the role of compartmentation in regulation of metabolism
9. How hormonal regulation control carbohydrate metabolism?

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I MSc BIOCHEMISTRY-Second Semester

REGULATION OF METABOLIC PATHWAYS (18BCP201)

UNIT-I **MULTIPLE CHOICE QUESTIONS**

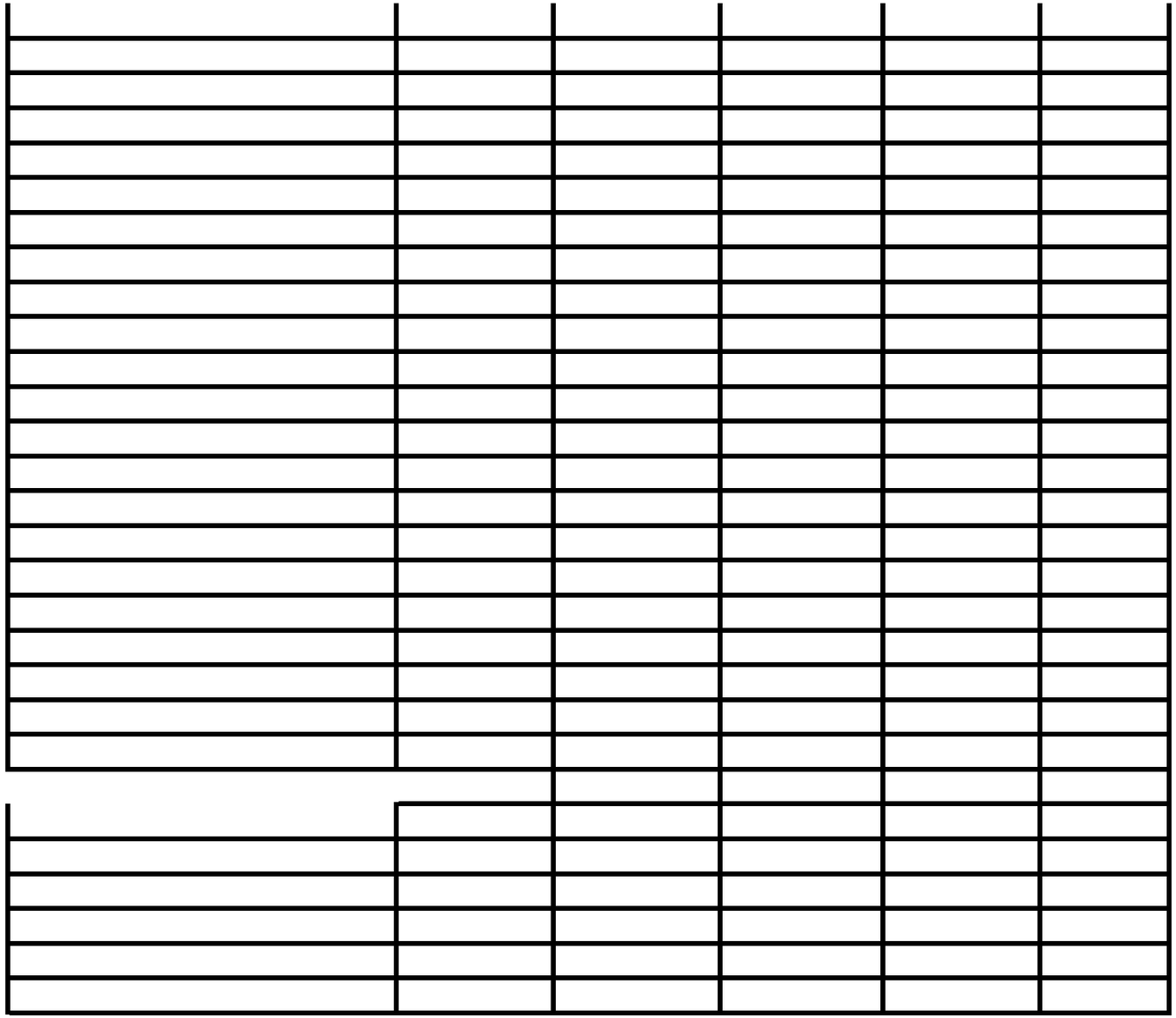
S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	The energy derived by oxidation of food is channeled into formation of _____	GTP	TTP	CTP	ATP	Answer
2	The committed step in each metabolic path way _____-	serves as an intermediate in the biosynthesis	proceeds with the large loss in the free energy	no role in the bio synthesis of end products	proceeds with the loss in the free energy	serves as an intermediate in the
3	The catalytic activity of regulatory enzymes are modulated by all except_____	allosteric control	covalent modification	phosphorylation and dephosphoryla	reduction	reduction
4	In the end product inhibition , the product act as a _____-	(+) ve modulator of	(-) ve modulator of	(+)ve (-)ve modulator	inert substance	(-) ve modulator
5	Synthesis of adenylate cyclase is increased by _____	thyroid hormones	growth hormones	ACTH	FSH	thyroid hormones
6	Metabolic path way are regulated by all except _____-	accessibility of substrate	amount of enzyme	control of enzymes	Cell growth	Cell growth
7	The number of ATP produced from oxidation of NADH is	2	3	1	4	3
8	Which one of the statement is not characteristics of catabolic reactions _____	divergent process	they serve to generate energy	they often produce NADH	they involve hydrolysis of macromolecu	divergent process
9	Compared to resting state , vigorously contracting muscle shows _____-	an decreased oxidation of pruvate to	an increased in conversion of pyruvate	a decreased NADH/NADR+ ratio	a decreased concentration of AMP	a decreased concentration of AMP

10						
11	Regulatory enzymes have the characteristic properties except _____	catalyses early step in a metabolic	follow michaelis menten	made up of more than one sub unit	show (+)ve or negative cooperativity	follow michaelis menten
12	Allosteric enzyme display _____	sigmoidal plots	hyperbolic plots	Linear plots	none of the above	sigmoidal plots
13	Radiolabelled compounds _____ the sensitivity of detection of the intermediate in the pathway.	increase	decrease	influence	do not change	increase
14	_____ is the source of ATP in heterotrophs.	sunlight	oxidation of organic	oxidation of inorganic	oxidation of organic and	oxidation of organic
15	which of the following is not a high energy compound?	ATP	creatine phosphate	phosphoenol pyruvate	glu- 6-phosphate	glu- 6-phosphate
16	C14 labeled compounds are used in the analysis of _____	amino acid metabolism	nucleic acid metabolism	carbohydrate metabolism	all the above.	4
17	_____ enzyme is inactivated through	Phosphorylase	phosphorylase kinase	glycogen synthase	pyruvate carboxylase	glycogen synthase
18	_____ enzyme is controlled through disulfide reduction.	thioredoxin reductase	citrate lyase	pyruvate carboxylase	pyruvate dehydrogenas	thioredoxin reductase
19	Enzyme controlled through adenylation is _____	Phosphorylase	glycogen synthase	glutamine synthase	pyruvate carboxylase	glutamine synthase
20	Except _____ all the others are controlled though proteolysis.	Kallikerin	Fibrinogen	Chymotrypsinogen	glutamine synthase	glutamine synthase
21	Number of cyclic AMP molecules required to activate cyclicAMP dependent protein kinase.	1	2	3	4	4
22	Glutamine synthatase regulation is achieved by	cumulative fed back	sequential feed back inhibition	enzyme multiplicity	conserted feed back	cumulative fed back

23	compartmentation limits the availability of _____	substrate	enzyme	co-factor	co-enzyme	substrate
24	Alpha- KG to sucinyl coA is inhibited by --------_____	fluoro acetate	arsenite	fluoride	iodo acetate.	arsenite
25						
26	P32 labelled compounds are used in the analysis of _____	amino acid metabolism	nucleic acid metabolism	carbohydrate metabolism	all the above	nucleic acid
27	S35 labeled compounds are used in the analysis of _____	amino acid metabolism	nucleic acid metabolism	carbohydrate metabolism	all the above	amino acid metabolis
28	Geiger muller counter working is based on _____-	proportional counting	liquid scintillation	auto radiography	fluoroscence counting	proportion al counting
29	The unit of radio activity is	curie	reontgen	rem	rad	curie
30	Enzyme controlled through uridylation is _____	Phosphorylas e	glycogen synthase	glutamine synthase	adenylate transferase	adenylate transferase
31	Citrate to Isocitrate is inhibited by --------_____	fluoro acetate	arsenite	fluoride	iodo acetate.	fluoro acetate
32	In glycolysis , enolase is inhibited by _____	iodoacetate	arsenite	flouride	flouroacetate	flouride
33	In glycolysis , glyceraldehydes 3 phosphate to 1,3bisphospho glycerate is inhibited by	iodoacetate	arsennite	flouride	flouroacetate	iodoacetate
34	Synthesis of tyrosine is a	Single step	Multistep	Branched	None	Single step
35	Structural analogue of lactose is	IPTG	BAP	Glucose	Mannose	IPTG
36	Among the following which one will alter the enzyme activity	Induction	Repression	Compartment ation	Covalent modification	Covalent modificatio
37	Among the following which will alter the enzyme activity	Covalent modification	allosteric modification	Both	None	3
38	Among the following which is the example for compartmentation	Glycolysis	Gluconeogene sis	TCA cycle	Glycogenesis	Gluconeog enesis

39	Citrate lyase is controlled through	Phosphorylati	Adenylation	Uridylation	Disulfide	Phosphoryl
40	HMGCoA reductase is controlled through	Phosphorylati on	Adenylation	Uridylation	Disulfide reduction	Phosphoryl ation
41	Phenyl alanine hydroxylase is controlled through	Phosphorylati on	Adenylation	Uridylation	Disulfide reduction	Phosphoryl ation
42						
43	Gleceraldehyde 3 phosphate dehydrogenase is controlled	Phosphorylati on	Adenylation	Uridylation	Disulfide reduction	Disulfide reduction
44	Glutamine synthetase is controlled through	Phosphorylati on	Adenylation	Uridylation	Disulfide reduction	Adenylatio n
45	Adenylate transferase is controlled through	Phosphorylati on	Adenylation	Uridylation	Disulfide reduction	Uridylation
46	Enzyme involved in blood cloting is	Trypsinogen	pepsin	Plasminogen	Chymotrypsi	Plasminog
47	Enzyme involved in programmed cell death	Elastase	pepsin	Collagenase	Carboxy peptidase	Collagenas e
48	Kallikrein is involved in	Programmed celldeath	Blood clotting	Dissolving blood clotting	Digestion	Blood clotting
49	Glycogen synthase is active in	Phosphorylat ed form	Dephosphoryl ated form	Both	None	Dephospho rylated
50	Glycogen Phosphorylase is active in	Phosphorylat ed form	Dephosphoryl ated form	Both	None	Phosphoryl ated form
51	Both substrate and effector molecules are same in	Homotrophic modifier	Heterotrophic modifier	Positive modifier	negative modifier	Homotroph ic modifier
52	Both substrate and effector moleculee are different in	Homotrophic modifier	Heterotrophic modifier	Positive modifier	negative modifier	Heterotrop hic
53	Inhibition of hexokinase by Glucose 6phosphate is an example	Homotrophic modifier	negative modifier	Positive modifier	none	negative modifier
54	Positive modifier of protein kinase	ATP	cAMP	AMP	All	**cAMP**

55	All are characteristic of Allosteric enzymes except	Control branched	Long term regulation	Control first commited step	Have cooperative	**Long term regulation**
56	Frutose1,6diphosphate is (feed forward) stimulator of	Enolase	Pyruvate dehydrogenase	Pyruvate carboxylase	Pyruvate kinase	Pyruvate kinase
57	Ability of each organelle to concentrate specific substance is	Allosteric modification	Covalent modification	Compartmentation	Proteolysis	Compartmentation
58	Ability of each organelle to create microenvironment is achieved by	Allosteric modification	Covalent modification	Compartmentation	Proteolysis	Compartmentation
59	Find the stable isotope in the list	C14	HJ3	Co60	N15	N15
60	Pathway analysis can be done in all the process except	Isotope	Complementation analysis	Genetic manipulation	Compartmentation	Compartmentation



UNIT-II

SYLLABUS

Carbohydrate Metabolism: An overview of Glycolysis and Gluconeogenesis. Role of LDH. Regulation of Glycolysis and Gluconeogenesis-Reciprocal control of Glycolysis and Gluconeogenesis, TCA cycle- steps, regulation at branch points; Glycogen Metabolism: Overview of glycogenesis and glycogenolysis. Reciprocal control of glycogenesis and glycogenolysis. Alternative pathways of metabolism-HMP shunt, Entner- doudoroff pathway, glucoronate and Glyoxalate pathway, cori cycle. Hormonal regulation of fuel metabolism; Metabolic disorders-Diabetes mellitus and insipidus.

Introduction to Glycolysis:

The most pressing need of all cells in the body is for an immediate source ofenergy. Some cells such as brain cells have severely limited storage capacities for either glucose or ATP, and for this reason, the blood must maintain a fairly constant supply of glucose. Glucose is transported into cells as needed and once inside of the cells, the energy producing series of reactions commences. The three major carbohydrate energy producing reactions are glycolysis, the citric acid cycle, and the electron transport chain.

The overall reaction of glycolysis which occurs in the cytoplasm is represented simply as:

$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P$ -----> 2 pyruvic acid, $(CH_3(C=O)COOH + 2\ ATP + 2\ NADH + 2\ H^+$

The major steps of glycolysis are outlined in below. There are a variety of starting points for glycolysis; although, the most usual ones start with glucose or glycogen to produce glucose-6-phosphate. The starting points for other monosaccharides, galactose and fructose, are also shown.

Important facts about glycolysis which are illustrated in the graphic.

1) Glucose Produces Two Pyruvic Acid Molecules:

2) ATP Is Initially Required:

3) ATP is Produced:

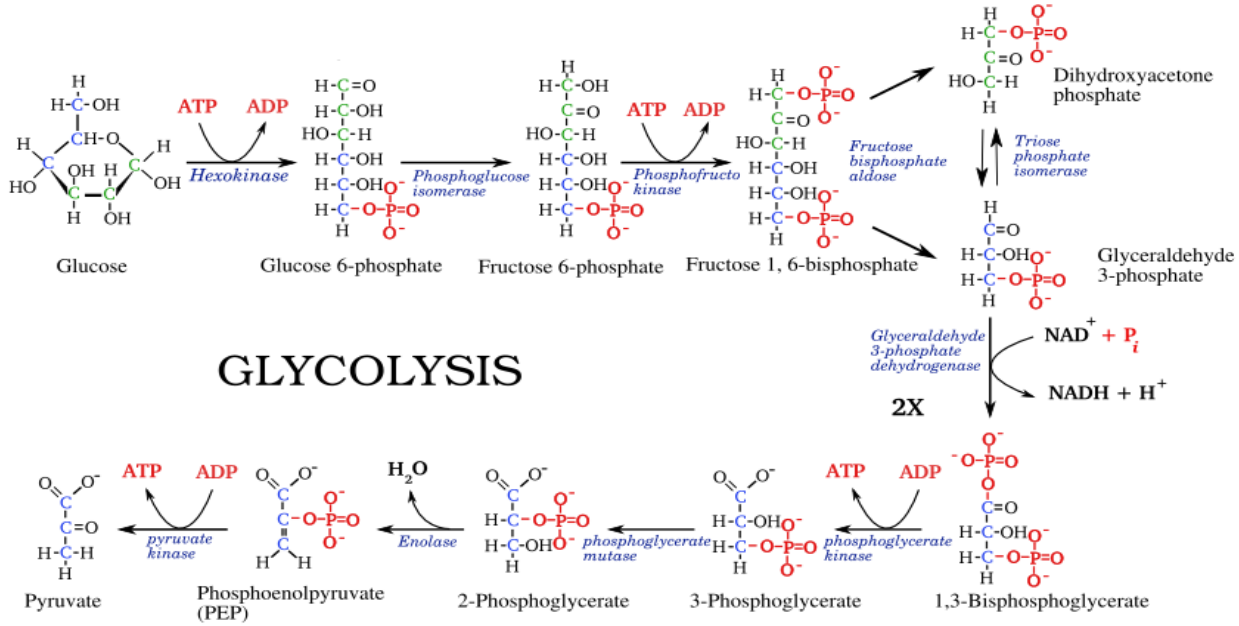
4) Fate of NADH + H^+:

If the cell is operating under aerobic conditions (presence of oxygen), then NADH must be reoxidized to NAD^+ by the electron transport chain. This presents a problem since glycolysis occurs in the cytoplasm while the respiratory chain is in the mitochondria which has membrane that is not permeable to NADH. This problem is solved by using glycerol phosphate as a "shuttle." The hydrogens and electrons are transferred from NADH to glycerol phosphate which can diffuse through the membrane into the mitochondria. Inside the mitochondria, glycerol phosphate reacts with FAD coenzyme in enzyme complex 2 in the electron transport chain to make dihydroxyacetone phosphate which in turn diffuses back to the cytoplasm to complete the cycle.

As a result of the the indirect connection to the electron transport at FAD, only 2 ATP are made per NAD used in step 5. If step 6 is used twice per glucose, then a total of **4 ATP** are made in this manner.

If the cell is anaerobic (absence of oxygen), the NADH product of reaction 5 is used as a reducing agent to reduce pyruvic acid to lactic acid at step 10. This results in the regeneration of NAD^+ which returns for use in reaction 6.

Steps in Glycolysis

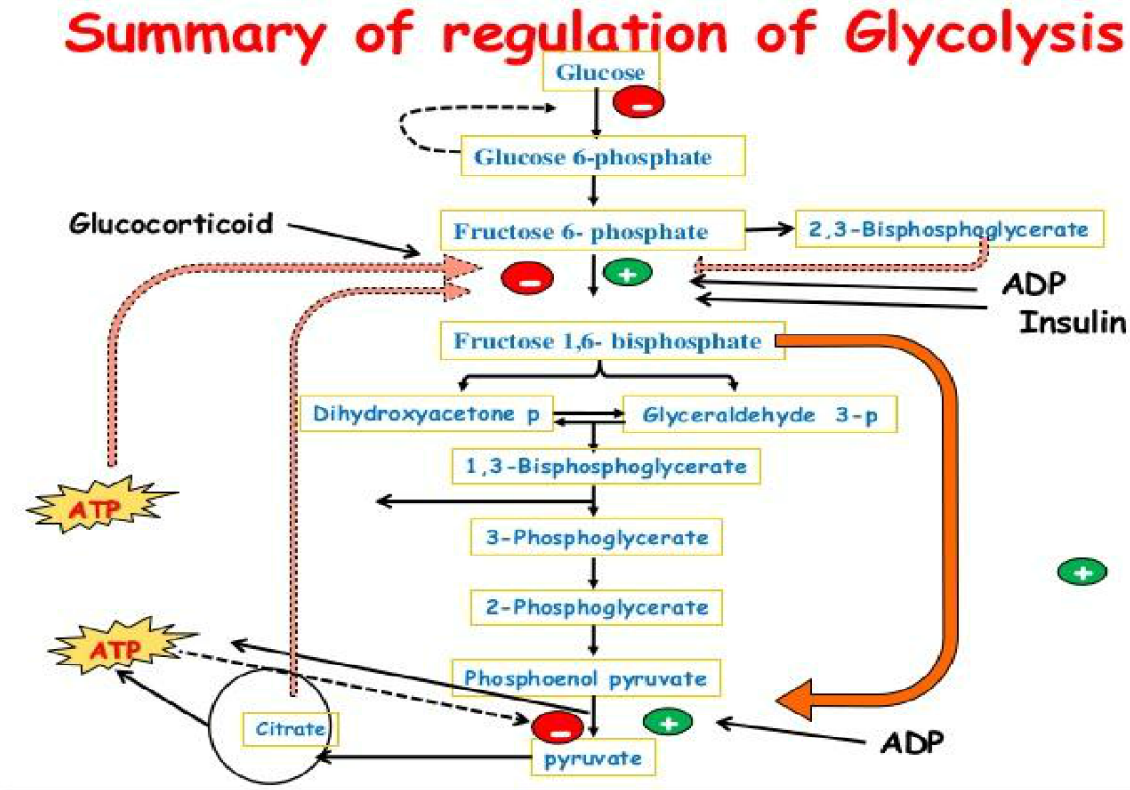


Regulation of Glycolysis:

Flux through a metabolic pathway can be regulated in several ways:

1. Availability of substrate
2. Concentration of enzymes responsible for rate-limiting steps
3. Allosteric regulation of enzymes
4. Covalent modification of enzymes (e.g. phosphorylation)

Of the 10 steps in the glycolytic pathway, three involve large negative free energy and are essentially irreversible. These are steps 1 (phosphorylation of glucose), 3 (phosphorylation of fructose-6-phosphate) and 10 (transfer of phosphate from phosphoenolpyruvate to ADP). These three enzymes are rate limitinf and their control is given below.



Of the three steps, steps catalysed by PFK is true rate limiting enzyme because hexokinase catalysed end product, glucose 6 phosphate can also be obtained from glogenolysis. So PFK is the true regulator of glycolysis

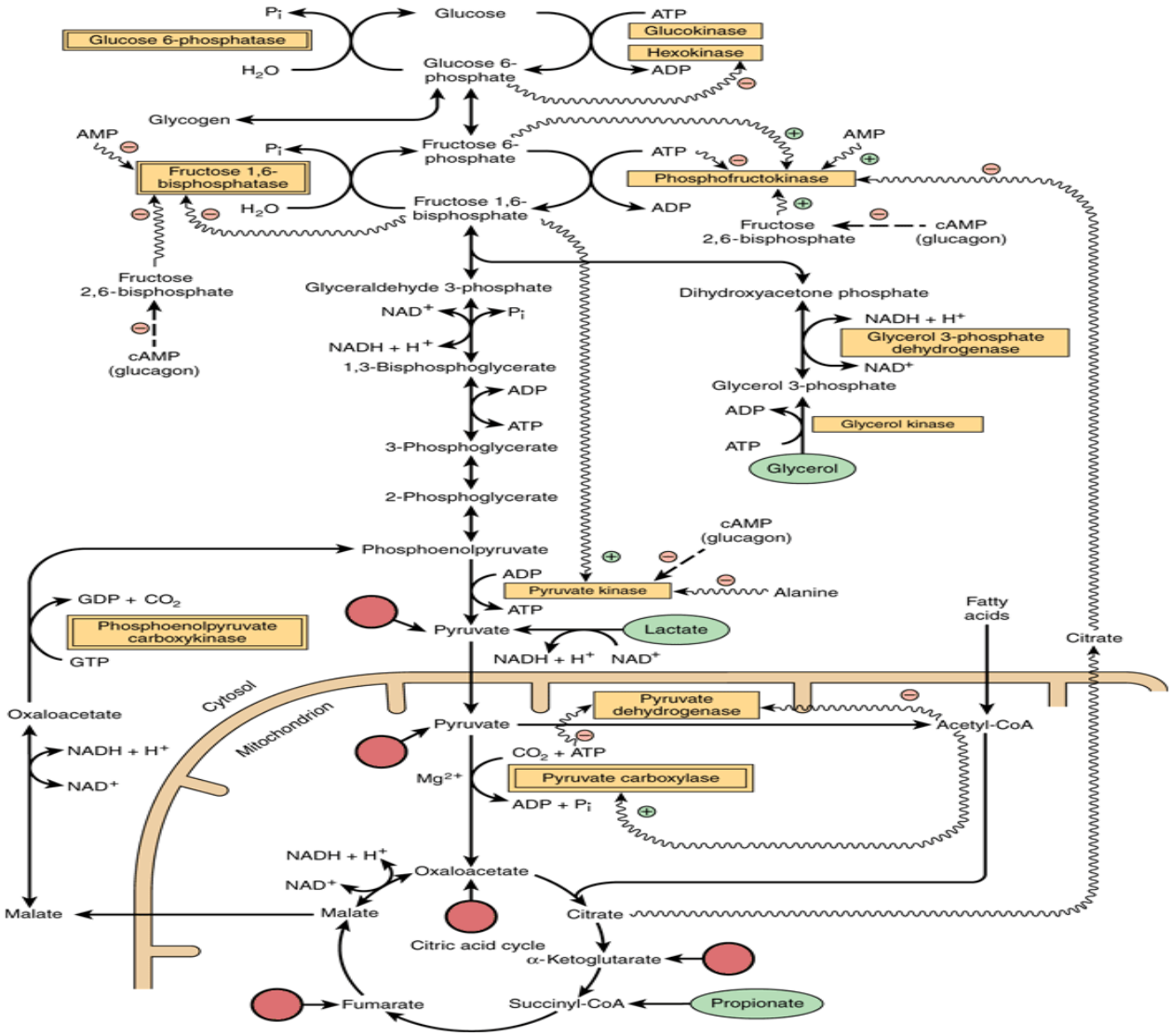
GLUCONEOGENESIS

Gluconeogenesis sources:

The **source of pyruvate and oxaloacetate** for gluconeogenesis during fasting or carbohydrate starvation is mainly **amino acid catabolism**. Some amino acids are catabolized to pyruvate, oxaloacetate, or precursors of these. Muscle proteins may break down to supply amino acids. These are transported to liver where they are deaminated and converted to gluconeogenesis inputs.

Glycerol, derived from hydrolysis of triacylglycerols in fat cells, is also a significant input to gluconeogenesis.

Fig: Sources of non carbohydrate source for gluconeogenesis



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 29th Edition*: www.accessmedicine.com

Regulation of Glycolysis &Gluconeogenesis

Gluconeogenesis occurs mainly in **liver**. Gluconeogenesis occurs to a more limited extent in the kidney and small intestine under some conditions.

Glycolysis & Gluconeogenesis pathways are **both spontaneous**. If both pathways were simultaneously active within a cell it would constitute a "**futile cycle**" that would waste energy. Overall, each pathway may be summarized as follows (ignoring water & protons):

Glycolysis:

glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

Gluconeogenesis:

2 pyruvate + 2 NADH + 4 ATP +2 GTP → glucose + 2 NAD^+ + 4 ADP + 2 GDP + 6 P_i

Glycolysis yields 2 ~P bonds of ATP.

Gluconeogenesis expends 6 ~P bonds of ATP and GTP.

A **futile cycle** consisting of both pathways would waste **4 ~P bonds per cycle**.

To prevent this waste, Glycolysis and Gluconeogenesis pathways are **reciprocally regulated Local Control** includes reciprocal allosteric regulation by **adenine nucleotides**.

- **Phosphofructokinase** (Glycolysis) is inhibited by ATP and stimulated by AMP.
- **Fructose-1,6-bisphosphatase** (Gluconeogenesis) is inhibited by AMP.

This insures that when cellular ATP is high (AMP would then be low), glucose is not degraded to make ATP. When ATP is high it is more useful to the cell to store glucose as glycogen. When ATP is low (AMP would then be high), the cell does not expend energy in synthesizing glucose.

Global Control in **liver** cells includes reciprocal effects of a **cyclic AMP cascade**, triggered by the hormone **glucagon** when blood glucose is low. **Phosphorylation** of enzymes and regulatory proteins in liver by Protein Kinase A (cAMP-Dependent Protein Kinase) results in inhibition of glycolysis and stimulation of gluconeogenesis, making glucose available for release to the blood. Proteins relevant to these pathways that are phosphorylated by Protein Kinase A include:

- **Pyruvate Kinase**, a glycolysis enzyme that is **inhibited** when phosphorylated.
- **CREB** (cAMP response element binding protein) which activates, through other factors, transcription of the gene for **PEP Carboxykinase**, leading to **increased gluconeogenesis**.
- A **bi-functional enzyme** that makes and degrades an allosteric regulator, **fructose-2,6-bisphosphate**.

Key enzymes of Gluconeogenesis

- Key Enzymes (4) :
  1. Pyruvate Carboxylase (PC)
  2. PEP Carboxykinase (PEPCK)
  3. Fructose 1,6 Bis phosphatase
  4. Glucose -6- Phosphatase

RECIPROCAL REGULATION OF GLYCOLYSIS AND GLUCONEOGENESIS BY FRUCTOSE-2,6-BISPHOSPHATE:

- **Fructose-2,6-bisphosphate stimulates Glycolysis**.
  - Fructose-2,6-bisphosphate allosterically **activates** the Glycolysis enzyme **Phosphofructokinase**.
  - Fructose-2,6-bisphosphate also **activates transcription** of the gene for **Glucokinase**, the liver variant of Hexokinase that phosphorylates glucose to glucose-6-phosphate, the input to Glycolysis.
- **Fructose-2,6-bisphosphate** allosterically **inhibits** the **gluconeogenesis** enzyme **Fructose-1,6-bisphosphatase**.

Recall that **Phosphofructokinase**, the rate-limiting step of the Glycolysis pathway, is **allosterically inhibited by ATP**. At high concentration, ATP binds to a low affinity regulatory site, promoting the tense conformation. Sigmoidal dependence of reaction rate on [fructose-6-phosphate] is observed at high ATP, as depicted at right.

Phosphofructokinase activity in the presence of the globally controlled allosteric regulator **fructose-2,6-bisphosphate** is similar to that observed when [ATP] is low. Fructose-2,6-bisphosphate promotes the **relaxed** state, activating Phosphofructokinase even at relatively high [ATP]. Thus **activation by fructose-2,6-bisphosphate**, whose concentration fluctuates in response to external hormonal signals, **supersedes local control** by ATP concentration.

The allosteric regulator **fructose-2,6-bisphosphate** is synthesized and degraded by a **bi-functional enzyme** that includes two catalytic domains:

- **Phosphofructokinase-2** (**PFK2**) domain

fructose-6-phosphate + ATP → fructose-2,6-bisphosphate + ADP.

- **Fructose-Biosphosphatase-2** (**FBPase2**) domain catalyzes:

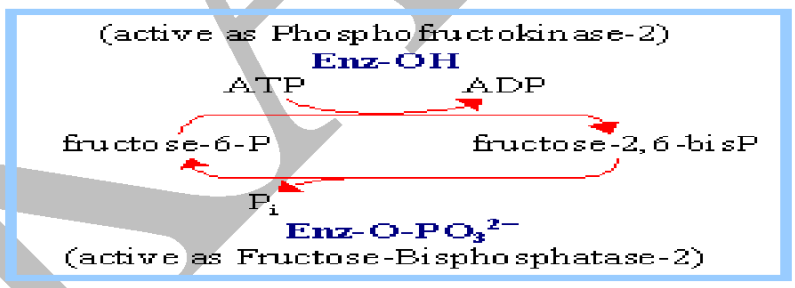
fructose-2,6-bisphosphate + H_2O → fructose-6-phosphate + P_i.

The bi-functional PFK2/FBPase2 assembles into a homodimer. Adjacent to the PFK2 domain in each copy of the liver enzyme is a **regulatory domain** subject to **phosphorylation** by cAMP-dependent Protein Kinase. Which catalytic domains of the enzyme are active depends on whether the regulatory domains are phosphorylated, as summarized below right.

cAMP-dependent **phosphorylation** of the bi-functional enzyme **activates FBPase2** and **inhibits PFK2**.

[**Fructose-2,6-bisphosphate**] thus **decreases** in liver cells in response to a cAMP signal cascade, activated by **glucagon** when blood glucose is low. Downstream effects include:

- **Glycolysis slows** because fructose-2,6-bisphosphate is not available to activate Phosphofructokinase.
- **Gluconeogenesis increases** because of the decreased concentration of fructose-2,6-bisphosphate, which would otherwise inhibit the gluconeogenesis enzyme Fructose-1,6-bisphosphatase.



Phosphorylation of the bifunctional enzyme is regulated by blood glucose level, mediated by glucagon and insulin. High glucagon (low blood sugar) causes phosphorylation of the enzyme, which results in conversion of F-2,6-BP back to F6P, removing its stimulatory effect on PFK, and therefore slowing the rate of glycolysis.

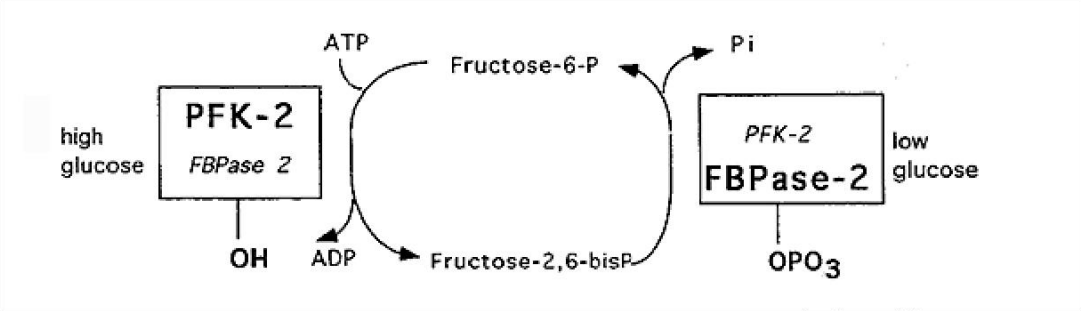


Fig: Bifunctional enzyme (phosphor fructo kinase 2 and fructose 2,6 bisphosphatase)

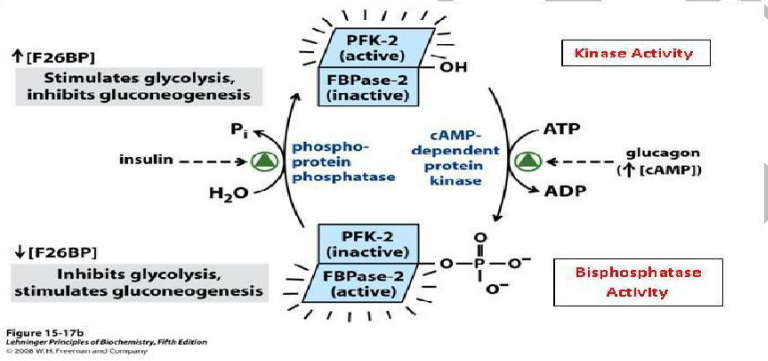


Fig: Bifunctional enzyme (phosphor fructo kinase 2 and fructose 2,6 bisphosphatase)

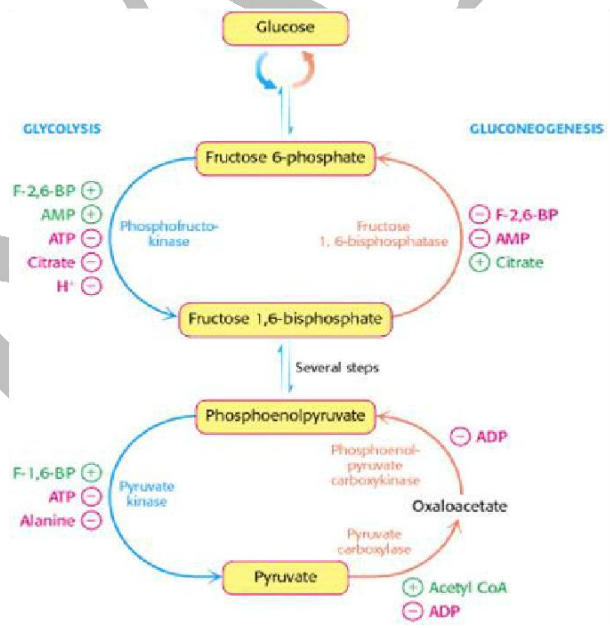


Fig: Regulators of glycolysis and gluconeogenesis

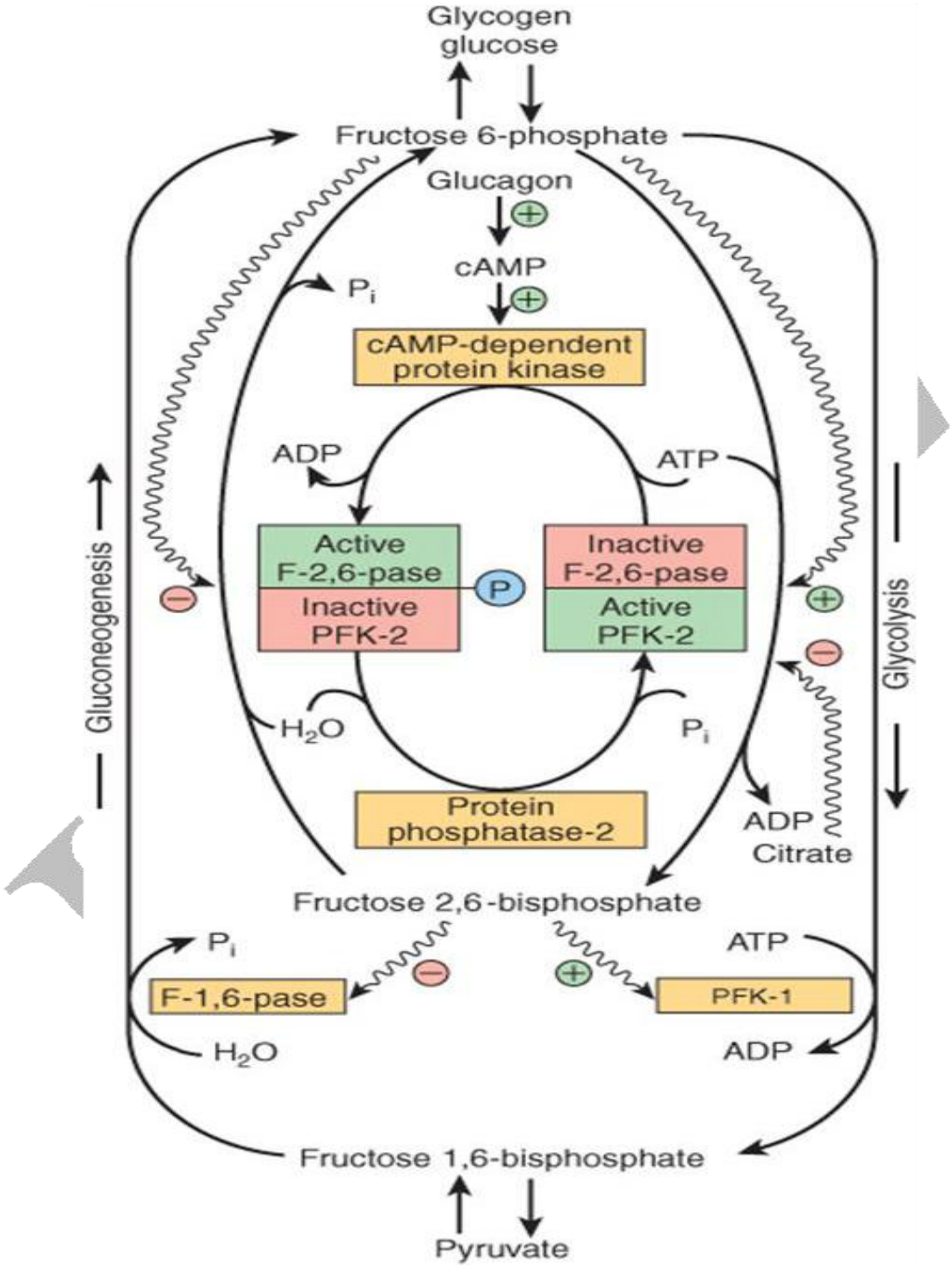


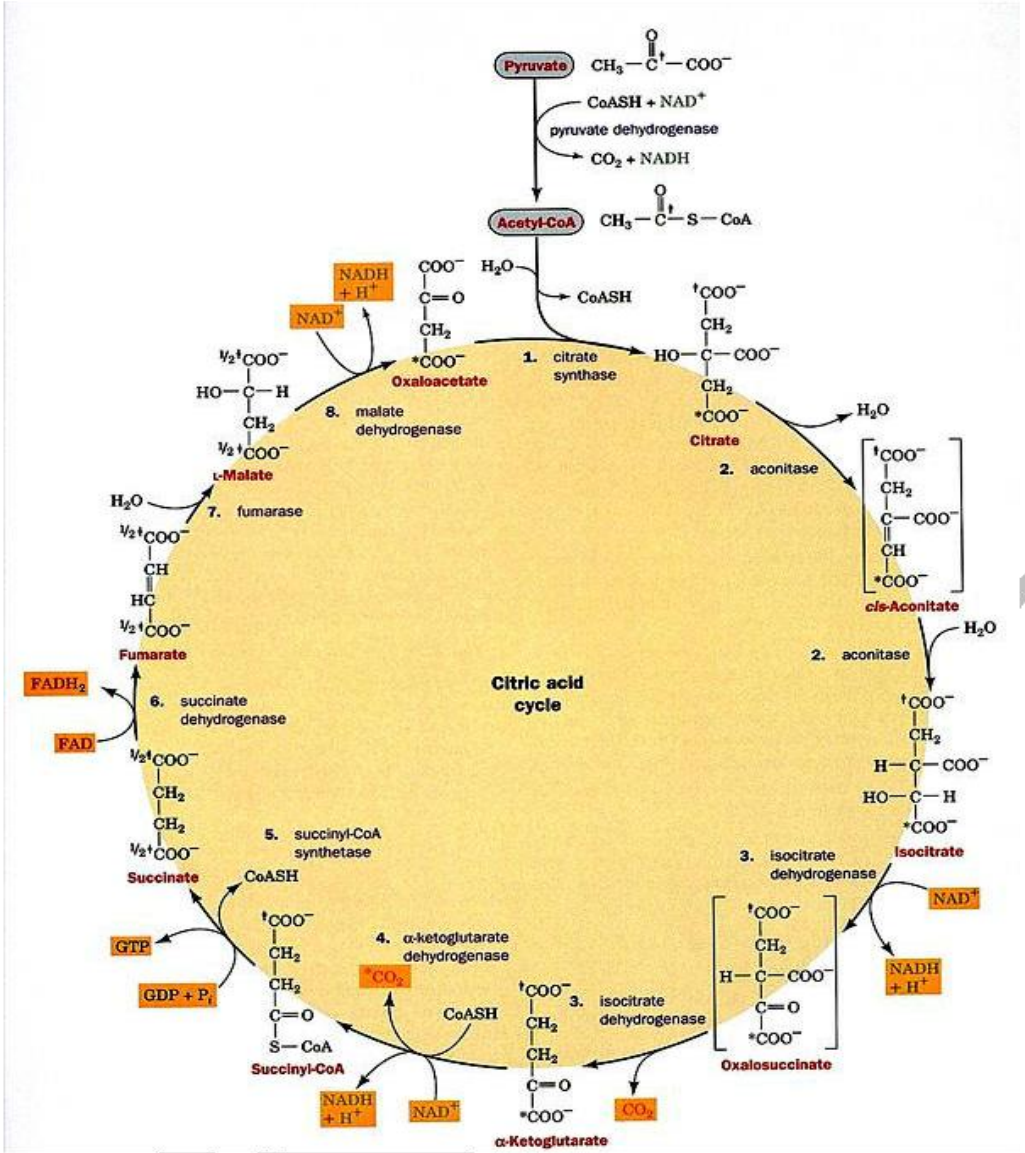
Fig: Reciprocal control of glycolysis and gluconeogenesis by fructose 2,6 bisphoosphate

CITRIC ACID CYCLE

Citric acid cycle— is a series of enzyme-catalysed chemical reactions, which is of central importance in all living cells, especially those that use oxygen as part of cellular respiration. In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. The components and reactions of the citric acid cycle were established by discovery of Vitamin C by Hungarian Nobel laureate Albert Szent-Györgyi and continued on to its complex metabolism into energy and metabolites by Nobel laureate Hans Adolf Krebs, a German born, Jewish refugee to Britain.

In aerobic organisms, the citric acid cycle is part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy. Other relevant reactions in the pathway include those in glycolysis and pyruvate oxidation before the citric acid cycle, and oxidative phosphorylation after it. In addition, it provides precursors for many compounds including some amino acids and is therefore functional even in cells performing fermentation. Its centrality to many paths of biosynthesis suggest that it was one of the earliest formed parts of the cellular metabolic processes, and may have formed abiogenically.

Steps in TCA cycles are given below



Regulation

The regulation of the TCA cycle is largely determined by substrate availability and product inhibition. NADH, a product of all dehydrogenases in the TCA cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits succinyl-CoA synthetase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and

vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

Calcium is used as a regulator. It activates pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate,a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

Recent work has demonstrated an important link between intermediates of the citric acid cycle and the regulation of hypoxia-inducible factors (HIF). HIF plays a role in the regulation of oxygen homeostasis, and is a transcription factor that targets angiogenesis, vascular remodeling, glucose utilization, iron transport and apoptosis. HIF is synthesized consititutively, and hydroxylation of at least one of two critical proline residues mediates their interaction with the von Hippel Lindau E3 ubiquitin ligase complex, which targets them for rapid degradation. This reaction is catalysed by prolyl 4-hydroxylases. Fumarate and succinate have been identified as potent inhibitors of prolyl hydroxylases, thus leading to the stabilisation of HIF.

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 groups 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.

GLYCOGEN 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

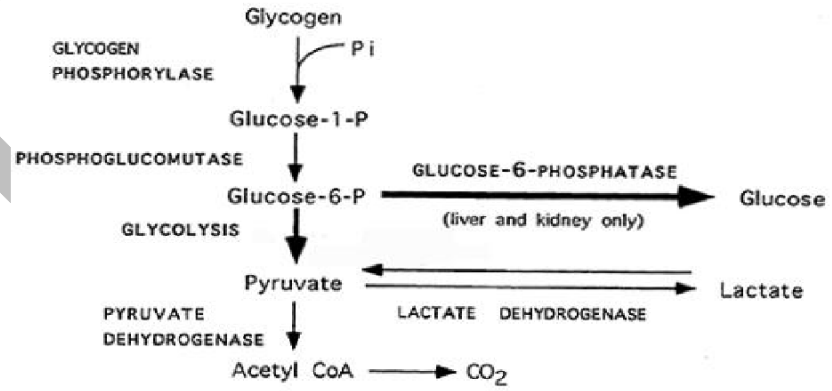
Glycogenolysis is the breakdown of glycogen (n) to glucose-1-phosphate and glycogen (n-1). Glycogen branches are catabolized by the sequential removal of glucose monomers via phosphorolysis, by the enzyme glycogen phosphorylase

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.

Reciprocal control of Glycogen Phosphorylase and Glycogen synthase:

Glycogen Phosphorylase in muscle is subject to allosteric regulation by AMP, ATP, and glucose-6-phosphate. A separate isozyme of Phosphorylase expressed in liver is less sensitive to these allosteric controls.

- **AMP** (present significantly when ATP is depleted) **activates** Phosphorylase, promoting the relaxed conformation.
- **ATP** and **glucose-6-phosphate**, which both have binding sites that overlap that of AMP, **inhibit** Phosphorylase, promoting the tense conformation.
- Thus glycogen breakdown is inhibited when ATP and glucose-6-phosphate are plentiful.

Glycogen Synthase is allosterically **activated by glucose-6-phosphate** (opposite of the effect on Phosphorylase). Thus Glycogen Synthase is active when high blood glucose leads to elevated intracellular glucose-6-phosphate.

It is useful to a cell to store glucose as glycogen when the input to Glycolysis (glucose-6-phosphate), and the main product of Glycolysis (ATP), are adequate.

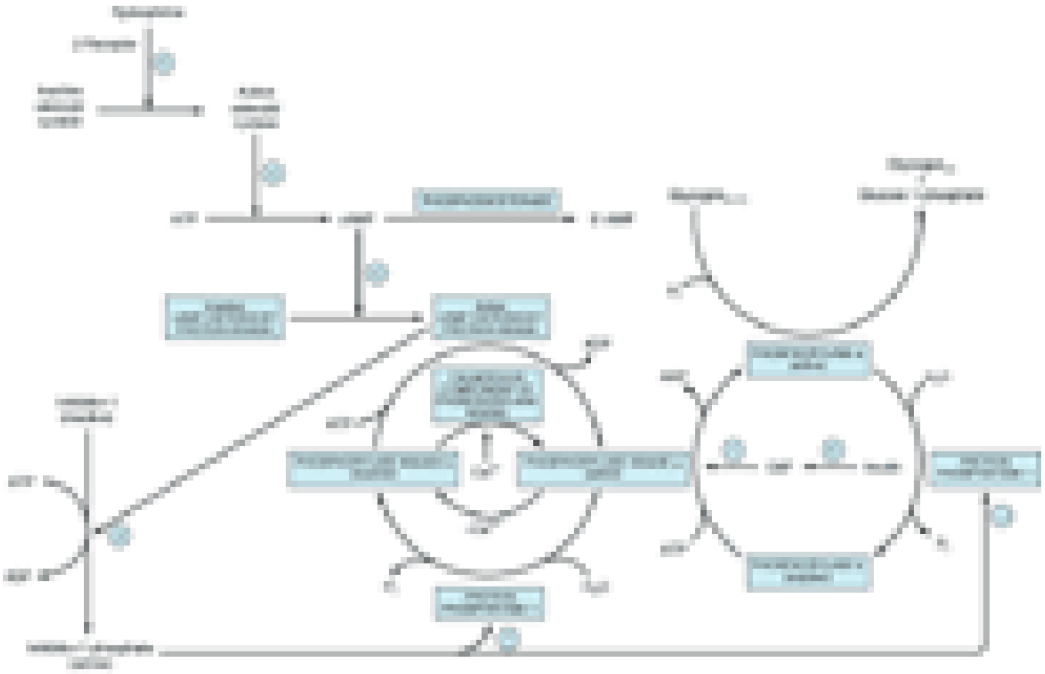


Fig: Reciprocal control of glycogenesis and glycogenolysis **HORMONAL REGULATION OF FUEL METABOLISM**

The hormones **glucagon** and **epinephrine** activate G-protein coupled receptors to trigger **cAMP cascades**. (cAMP is discussed in the section on cell signals). Both hormones are produced in response to **low blood sugar**. Glucagon, which is synthesized by -cells of the pancreas, activates cAMP formation in liver. Epinephrine activates cAMP formation in muscle. The **cAMP** cascade results in **phosphorylation** of a serine hydroxyl of the **Glycogen Phosphorylase** enzyme, which promotes transition to the **active** (relaxed) state. The phosphorylated enzyme is less sensitive to allosteric inhibitors. Thus even if cellular ATP and glucose-6-phosphate are high, Phosphorylase will be active. The glucose-1-phosphate produced from glycogen in liver may be converted to free glucose for release to the blood. With this hormone-activated regulation by covalent modification, the needs of the organism take precedence over the needs of the cell.

: The **cAMP cascade** has the **opposite effect on glycogen synthesis**. Glycogen Synthase is phosphorylated by Protein Kinase A as well as by Phosphorylase Kinase. **Phosphorylation of Glycogen Synthase promotes the "b" (less active) conformation**. The cAMP cascade thus inhibits glycogen synthesis. Instead of being converted to glycogen, glucose-1-phosphate in liver may be converted to glucose-6-phosphate, and dephosphorylated for release to the blood.

High cytosolic glucose-6-phosphate, which would result when blood glucose is high, turns off the signal with regard to glycogen synthesis. The conformation of Glycogen Synthase induced by the allosteric activator glucose-6-phosphate is susceptible to dephosphorylation by Protein Phosphatase.

Insulin, produced in response to **high blood glucose**, triggers a separate signal cascade that leads to **activation of Phosphoprotein Phosphatase**. This phosphatase catalyzes removal of regulatory phosphate residues from Phosphorylase, Phosphorylase Kinase, and Glycogen Synthase enzymes. Thus **insulin antagonizes** effects of the cAMP cascade induced by **glucagon** and **epinephrine**.

Ca^{++} also regulates glycogen breakdown in **muscle**. During activation of contraction in skeletal muscle, Ca^{++} is released from the sarcoplasmic reticulum to promote actin/myosin interactions.

Summarizing effects described above and in the notes on glycogen metabolism, a glucagon-induced **cAMP cascade** has the following effects in liver tissue:

- Gluconeogenesis is stimulated.
- Glycolysis is inhibited.
- Glycogen breakdown is stimulated.
- Glycogen synthesis is inhibited.
- Free glucose formed for release to the blood.

Metabolic disorder

A **metabolic disorder** can happen when abnormal chemical reactions in the body alter the normal metabolic process. It can also be defined as inherited single gene anomaly, most of which are autosomal recessive.

Diabetes Overview

Diabetes is a number of diseases that involve problems with the hormone insulin. Normally, the pancreas (an organ behind the stomach) releases insulin to help your body store and use the sugar and fat from the food you eat. Diabetes can occur when the pancreas produces very little or no insulin, or when the body does not respond appropriately to insulin. As yet, there is no cure. People with diabetes need to manage their disease to stay healthy.

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes.

All types of diabetes mellitus have something in common. Normally, your body breaks down the sugars and carbohydrates you eat into a special sugar called glucose. Glucose fuels the cells in your body. But the cells need insulin, a hormone, in your bloodstream in order to take in the glucose and use it for energy. With diabetes mellitus, either your body doesn't make enough insulin, it can't use the insulin it does produce, or a combination of both.

Since the cells can't take in the glucose, it builds up in your blood. High levels of blood glucose can damage the tiny blood vessels in your kidneys, heart, eyes, or nervous system. That's why diabetes -- especially if left untreated -- can eventually cause heart disease, stroke, kidney disease, blindness, and nerve damage to nerves in the feet.

Type 1 Diabetes

Type 1 diabetes is also called insulin-dependent diabetes. It used to be called juvenile-onset diabetes, because it often begins in childhood. It is an autoimmune condition. It's caused by the body attacking its own pancreas with antibodies. In people with type 1 diabetes, the damaged pancreas doesn't make insulin.

This type of diabetes may be caused by a genetic predisposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin.

A number of medical risks are associated with type 1 diabetes. Many of them stem from damage to the tiny blood vessels in your eyes (called diabetic retinopathy), nerves (diabetic

neuropathy), and kidneys (diabetic nephropathy). Even more serious is the increased risk of heart disease and stroke.

Treatment for type 1 diabetes involves taking insulin, which needs to be injected through the skin into the fatty tissue below. The methods of injecting insulin include:

- Syringes
- Insulin pens that use pre-filled cartridges and a fine needle
- Jet injectors that use high pressure air to send a spray of insulin through the skin
- Insulin pumps that dispense insulin through flexible tubing to a catheter under the skin of the abdomen

Type 2 Diabetes

By far, the most common form of diabetes is type 2 diabetes, accounting for 95% of diabetes cases in adults. Some 26 million American adults have been diagnosed with the disease. Type 2 diabetes used to be called adult-onset diabetes, but with the epidemic of obese and overweight kids, more teenagers are now developing type 2 diabetes. Type 2 diabetes was also called non-insulin-dependent diabetes.

Type 2 diabetes is often a milder form of diabetes than type 1. Nevertheless, type 2 diabetes can still cause major health complications, particularly in the smallest blood vessels in the body that nourish the kidneys, nerves, and eyes. Type 2 diabetes also increases your risk of heart disease and stroke.

With Type 2 diabetes, the pancreas usually produces some insulin. But either the amount produced is not enough for the body's needs, or the body's cells are resistant to it. Insulin resistance, or lack of sensitivity to insulin, happens primarily in fat, liver, and muscle cells.

People who are obese -- more than 20% over their ideal body weight for their height -- are at particularly high risk of developing type 2 diabetes and its related medical problems. Obese people have insulin resistance. With insulin resistance, the pancreas has to work overly hard to produce more insulin. But even then, there is not enough insulin to keep sugars normal.

There is no cure for diabetes. Type 2 diabetes can, however, be controlled with weight management, nutrition, and exercise. Unfortunately, type 2 diabetes tends to progress, and diabetes medications are often needed.

Glycogen Storage Disease (GSD)

A **glycogen storage disease** (GSD, also glycogenosis and dextrinosis) is a metabolic **disorder** caused by enzyme deficiencies affecting either **glycogen** synthesis, **glycogen** breakdown or glycolysis (glucose breakdown), typically within muscles and/or liver cells. GSD has two classes of cause: genetic and acquired. Genetic GSD is caused by any inborn error of metabolism (genetically defective enzymes) involved in these processes. In livestock, acquired GSD is caused by intoxication with the alkaloid castanospermine.

The underlying problem in all of the Glycogen Storage Diseases is the use and storage of glycogen. Sometimes GSDs are also referred to as glycogenoses because they are caused by difficulty in glycogen metabolism.

All of the Glycogen Storage Diseases are considered inherited metabolic disorders. A metabolic disorder is a disease that disrupts metabolism. Therefore, a person who has a metabolic disorder has a difficult time breaking down certain foods and creating energy. A metabolic disease is most frequently caused by an absence or deficiency in an enzyme (or protein). An enzyme can act to help the body break down food into energy. There are many enzymes in the body and each act like a machine on an assembly line. When one of the enzymes is not working properly, the process of breaking down of specific foods can go more slowly or shut down completely.

A person with a glycogen storage disease (GSD) has an absence or deficiency of one of the enzymes responsible for making or breaking down glycogen in the body. This is called an enzyme deficiency. The enzyme deficiency causes either abnormal tissue concentrations of glycogen (too much or too little) or incorrectly or abnormally formed glycogen (shaped wrong). Depending on the type of GSD a person has, their enzyme deficiency may be important in all parts of the body, or only in some parts of

the body, like the liver or muscle. Typically, the forms of GSD are described by the part of the body that has trouble because of the enzyme deficiency. The categories most often are: the liver only, the muscles only, or both the liver and the muscles. Other systems that may be involved include blood cells (red blood cells, white blood cells, and platelets), heart, and kidneys amongst others.

All types of GSD cause the body to either not be able to make enough glucose, or not be able to use glucose as a form of energy. Determining what type of GSD a person has (diagnosis) depends on an individual's symptoms. Typically a doctor will do a physical examination and some blood and urine testing. Occasionally, a muscle and/or liver biopsy will be needed to measure the amount of a certain enzyme in that part of the body.

There are about eleven known types of GSD, which are classified by a number, by the name of the defective enzyme, or by the name of the doctor who first described the condition. For example, Glycogen Storage Disease Type Ia, caused by a defect in the enzyme glucose-6-phosphatase, was originally known as ―von Gierke's Disease‖ (after Edgar von Gierke, the doctor who discovered it) but is also referred to as ―Glucose-6-Phosphatase Deficiency Glycogen Storage Disease.‖

The GSDs are genetic disorders. This means that they are caused by a change in a part of an individual’s genetic information. Our genetic information is stored on genes. Genes serve as the instruction manual for our bodies. They tell our bodies how to grow and function. They also determine our physical features, such as hair color and eye color. We have around 30,000 genes in every cell of our body. We get two sets of every gene, one set from our mother and one set from our father. This is why we appear to be a combination of our parents. Our parents have no control over which genes they pass on to us. The genes we inherit from our parents happen purely by chance.

If there is a change in the genetic information contained on one of these genes, our bodies are unable to read its instructions. Therefore, it may cause a difference in the way our body functions. This is similar to having a page missing out of an instruction manual for putting an appliance together. Without that page, we would be unable to properly assemble the appliance and it would not be able to work. Almost all forms of GSD occur when a child inherits an incorrect genetic instruction from both their mother

and their father (autosomal recessive inheritance). Some forms of GSD are caused by a genetic change that is passed from mother to son (sex or X-linked inheritance).

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II Pompe	α-1,4-Glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure.	Cardiorespiratory failure causes death, usually before age 2.
III Cori	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV Andersen	Branching enzyme (α-1,4 → α-1,6)	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V McArdle	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI Hers	Phosphorylase	Liver	Increased amount.	Like type I, but milder course.
VII	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.

Treatment

Treatment is depended on the type of glycogen storage disease. E.g. GSD I is typically treated with frequent small meals of carbohydrates and cornstarch to prevent low blood sugar, while other treatments may include allopurinol and human granulocyte colony stimulating factor.

POSSIBLE QUESTIONS

2 mark questions

1. Explain the energetics of TCA cycle.
2. List the regulatory enzymes of Gluconeogenesis.
3. Add short notes on glycogen storage disease
4. Define the process glycogenesis and its importance
5. How many ATP molecules are produced by the aerobic oxidation of glycolysis?
6. Add notes on diabetes mellitus
7. Add short notes on hormone involved in carbohydrate metabolism
8. Short notes on regulation of TCA cycle
9. What is gluconeognesis? List the four important regulatory enzyme of it.
10. Enumerate the ATP production in aerobic glycolysis
11. Give the flow chart of TCA cycle.
12. Write the regulation of Pyruvate dehydrogenase

Essay type questions (6Marks)

1. Give the reactions of glycolysis
2. Explain the gluconeogenic process with neat flow chart
3. Explain the reciprocal control of glycogenesis and glycogenolysis
4. How glycogen synthesis occur in our body
5. Give the source and reactions of gluconeogenesis
6. Explain in detail about the reciprocal control of gluconeogenesis and glycolysis
7. Explain in detail about the reactions and control of gluconeogenesis
8. With neat diagram explain about the reactions and control of TCA cycle

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I MSc BIOCHEMISTRY-Second Semester

REGULATION OF METABOLIC PATHWAYS (18BCP201)

UNIT-II **MULTIPLE CHOICE QUESTIONS**

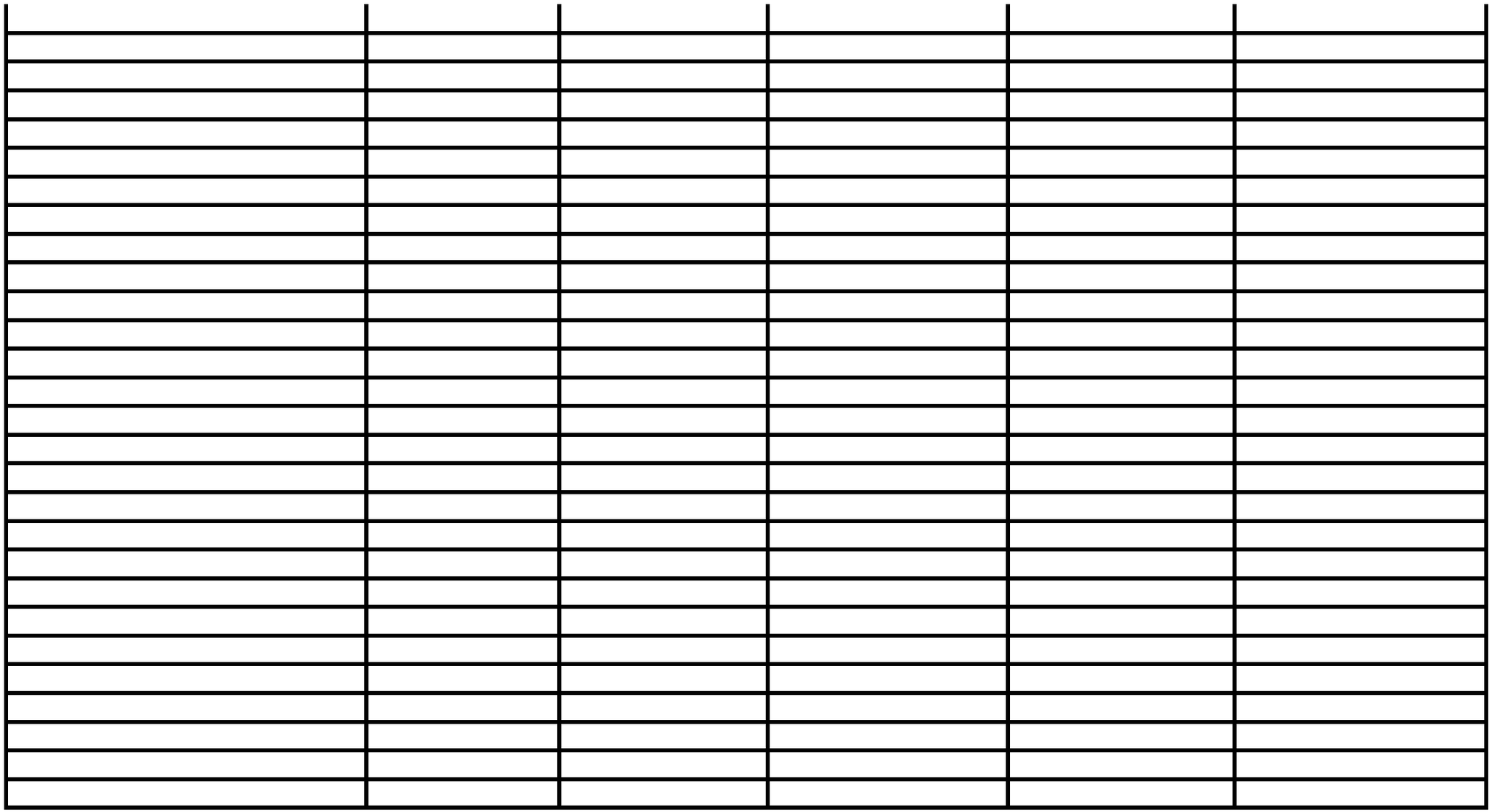
S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Formation of fructose -6-phosphate from glucose -6-phosphate is catalyzed by_____	phosphofructokinase	aldolase	hexo kinase	phosphorglucose isomerase	phosphorglucose isomerase
2	Animal cells can synthesis glucose from the following except _____	citrate	acetyl CoA	malonyl CoA	succinyl CoA	malonyl CoA
3	Allosteric enzyme stimulated by ADP	ATP-citrate lyase	isocitrate dehydrogenase	α-ketoglutarate dehydrogenase.	none of the above.	isocitrate dehydrogenase
4	The glyoxalate cycle by passes _____	Two decarboxylation steps of the TCA	one decarboxylation of the TCA	no carboxylation step of the TCA	none of the above	Two decarboxylation steps of the TCA
5	Pyruvate dehydrogenase is regulated by the _____	NAD+/NADH ratio	ATP/ADP ratio	CoA/acetylCoA	all the above	all the above
6	The soul source of energy for Brain obtained from	Malate	Glucose	Fatty acid	Ketone bodies	Glucose
7	Phosphorfructokinase is inhibited by _____	fructose2,6-bisphosphate	citrate	AMP	fructose-6-phosphate	citrate
8	In the krebs cycle NADH inhibits the following enzymes except_____	citrate synthase	aconitase	isocitrate dehydrogenase	α-ketoglutarate dehydrogenase	aconitase
9	Conversion of the fru6PO4 to fruc1,6, bis PO4 is catalyzed by the enzyme _____	aldose	phosphofructokinase	phosphoglyceratemutase	phosphoglycerate kinase	phosphofructokinase

10	Pyruvate dehydrogenase complex is covalently modified by _____	phosphorylation	methylation	acetylation	ADP-ribosylation	phosphorylation
11	The conversion of 3-phosphoglycerate into phosphophenol pyruvate is catalyzed by	hexokinase	glucokinase	fructokinase	enolase	enolase
12	Hexokinase is inhibited by _____	ATP	NADH	citrate	glucose-6-phosphate	glucose-6-phosphate
13	Glycogenesis occurs mainly in the _____	liver and kidney	kidney and muscle	muscle and liver._	liver and mitochondria	muscle and liver._
14	Which enzyme is involved in gluconeogenisis	Pyruvate kinase	Phosphoenolpyruvate carboxy kinase	Phosphorylase	Enolase	Phosphoenolpyruvate carboxy kinase
15	Phosphofructokinase is the regulatory enzyme for _____	cholesterol synthesis	glycolysis	fatty acid biosynthesis	purine biosynthesis	glycolysis
16	Glycolysis is regulated by the following enzymes except	Hexokinase	phospho fructokinase	phosphoglycerate kinase	pyruvate kinase	phosphoglycerate kinase
17	In glycogenolysis, the rate limiting step is catalyzed by _____	phosphorylase	amylo(1→6)glucosidase	Glucose-6-phosphatase	α-(1→4)→α-(1→4)glucan transferase	phosphorylase
18	Citric acid cycle take part in _____	gluconeogenosis	transamination	fatty acid oxidation	all the above	all the above
19	The following reactions are examples for substrate level phosphorylation expect ___	succinate thiokinase	phosphoglycerate kinase	pyruvate kinase	phosphophenolpyruvatecarboxy kinase	phosphophenolpyruvatecarboxy kinase
20	All the enzymes of glycolysis are found in the _____	cytosol	mitochondria	both	endoplasmic reticulum	cytosol
21	Which one of the following vitamin is not involved in the citric acid cycle .?	riboflavin	niacin	thiamine	ascorbic acid	ascorbic acid
22	In glycolysis , enolase is inhibited by _____	iodoacetate	arsennite	flouride	flouroacetate	flouride

23	Which of the following is a constituent of HMP shunt?	glucose-6-phosphatase	glucose-6-Phospate dehydrogenase._	hexo kinase	phosphorylase.	glucose-6-Phospate dehydrogenase._
24	Glucose oxidation is controlled by _____	phosphor fructo kinase	lipase	amylase	citrate synthetase	phosphor fructo kinase
25	Which one of the following inhibitor in TCA cycle acts as by blocking citrate?	arsenite	flouroacetate	malonate	malate	flouroacetate
26	How many ATP's formed in (aerobic) glycolysis ?	5	15	8	11	8
27	Sugar formed in HMP shuntand used for Nucleic acid synthesis	heptose	heptulose	Ribose	Gluose	Ribose
28	Glucose is removed from the blood following a meal_____	Hexokinase	glucokinase	both	none of the above	glucokinase
29	_____has affinity power to glucose	galacto kinase	fructokinase	glucokinase	all the above	glucokinase
30	Galactose is phosphorylated by galactokinase to form _____	galactose-1, 6-diphosphate	galactose-6-phosphate	galactose-1-phosphate	all the above	galactose-6-phosphate
31	UDPG oxidized to UDP-glucuronic acid by UDP dehydrogenase in presence of	NAD+	FAD+	NADP+	ADP+	NAD+
32	Glycogen synthetase is depressed by_____	glucose	insulin	fructokinase	cAMP	cAMP
33	Fructokinase is present in _____	intestine	adipose tissue	heart	brain	intestine
34	Hexo kinase enzyme _____-	requires Ca2+ for activity	catalyses the transfer of a phosphoryl group to a variety of hexose	catalyses the conversion of glucose-6po4 to fructose 1-6-di po4	catalyses a phosphoryl shift reaction	catalyses the transfer of a phosphoryl group to a variety of hexose
35	What happens when acetyl CoA is abundant_____	pyruvate carboxylase is activated	phosphoenol pyruvat carboxy kinase is activated	phospho fructo kinase is activated	pyruvate de hydrogenase is activated	pyruvate carboxylase is activated

36	Phosphofructo kinase is an allosteric enzyme activated by_____	concentration of ATP	citrate	long chain fatty acid	concentration of AMP	citrate
37	Which one of the following statement about Ca2+ is correct _____	intracellular concentration of Ca2+ is higher than that of extracellular fluid	concentration of cytosolic Ca2+ is decreased by activation of phospholipids choline	the effect of Ca2+ are often mediated by calmodulin	intracellular Ca2+ is mostly is free	the effect of Ca2+ are often mediated by calmodulin
38	The activity of glycogen phosphorylase is allosterically inhibited by _____	ATP	G-6-P	glucose	All the above	G-6-P
39	The activity of pyruvate kinase is induced by _____	insulin	glucagon	Epine phrine	Alanine	insulin
40	PEPCK activity is decreased in _____	carbohydrate feeding	starvation and diabetes	carbohydrate feeding and diabetes	none of the above	starvation and diabetes
41	The main product of glycolysis is skeletal musle under anaerobic condition is_____	Pyruvate	Glutamate	acetyl CoA	lactate	lactate
42	The functions of NAD+_____	e- transfer	p-transfer	acyl group transfer	phosphorylate most of the hexoses	e- transfer
43	One of the following is a rate limiting enzyme in gluconeogenesis _____	hexokinase	phosphofructokinase	pyruvate carboxylase	pyruvate kinase	pyruvate carboxylase
44	The negative modulator of hexokinase	Glucose	Fructose	glucose 6 phosphate	fructose 6 phosphate	glucose 6 phosphate
45	Anaerobic glycolysis yield_____ATP	**2**	8	12	24	1
46	Citrate to Isocitrate is inhibited by --------_____	fluoro acetate	arsenite	fluoride	iodo acetate.	fluoro acetate
47	In glycolysis , enolase is inhibited by _____	iodoacetate	arsenite	flouride	flouroacetate	flouride
48	In glycolysis , glyceraldehydes 3 PO4 to 1,3bisphospho glycerate is inhibited by	iodoacetate	arsennite	flouride	flouroacetate	iodoacetate

49	Alpha- KG to sucinyl coA is inhibited by --------_____	fluoro acetate	arsenite	fluoride	iodo acetate.	arsenite
50	The sources of glucose from gluconeogenesis is all the following except one	Glycogen	fatty acid	protein	lactate	Glycogen
51	Stimulated enzyme in Glycolysis	Hexokinase	phospho fructo kinase	pyruvate kinase	Enolase	pyruvate kinase
52	The following are inhibitor of frutose1,6 diphosphatase , except	AMP	Fructose 1,6 diphosphate	Fructose 2,6 diphosphate	ATP	ATP
53	Glucose 6 phosphatase is stimulated by following except	Glucagon	epinephrine	glucocorticoids	Insulin	Insulin
54	Positive modifier of frutose1,6 diphosphatase is	AMP	Fructose 1,6 diphosphate	Fructose 2,6 diphosphate	cAMP	cAMP
55	The Key molecule in reciprocalcontrol of glycolysis and gluconeogenesis is	Citrate	Fructose 1,6 diphosphat	Fructose 2,6 diphosphate	ATP	Fructose 2,6 diphosphate
56	Structural analogue of succinate is	Fumarate	malonate	malate	oxalo acetate	malonate
57	All are multi enzyme complex except	pyruvate dehydrogenase	pyruvate carboxylase	alpha keto dehydrogenase	fatty acid synthase complex	pyruvate carboxylase
58	Find the multi enzyme complex from the list given	pyruvate dehydrogenase	pyruvate carboxylase	pyruvate kinase	citrate synthase	pyruvate dehydrogenase
59	cAMP stimulates	glycogenesis	Glycogenolysis	glycolysis	TCA cycle	Glycogenolysis
60	cAMP is inactivated by	adenylate cyclase	phospho lipase	phospho diesterase	protein kinase	phospho diesterase



UNIT-III

SYLLABUS

Lipid metabolism: An overview of fatty acid synthesis and degradation, Regulation of fatty acid synthesis- control of acetyl CoA carboxylase and fatty acid synthetase complex; Reciprocal control of fatty acid synthesis and degradation. Biosynthesis of triacyl glycerol, phosphatidyl choline, phosphotidyl ethanolamine and sphingomyelin and their regulation. Synthesis and degradation of cholesterol and its regulation. Metabolism of prostaglandins-COX and LOX pathways. Metabolic fate of VLDL, LDL and HDL. Obesity and regulation of body mass. Metabolic disorders- Atherosclerosis, Hyper and hypo lipoproteinemia.

BIOSYNTHESIS OF SATURATED FATTY ACIDS

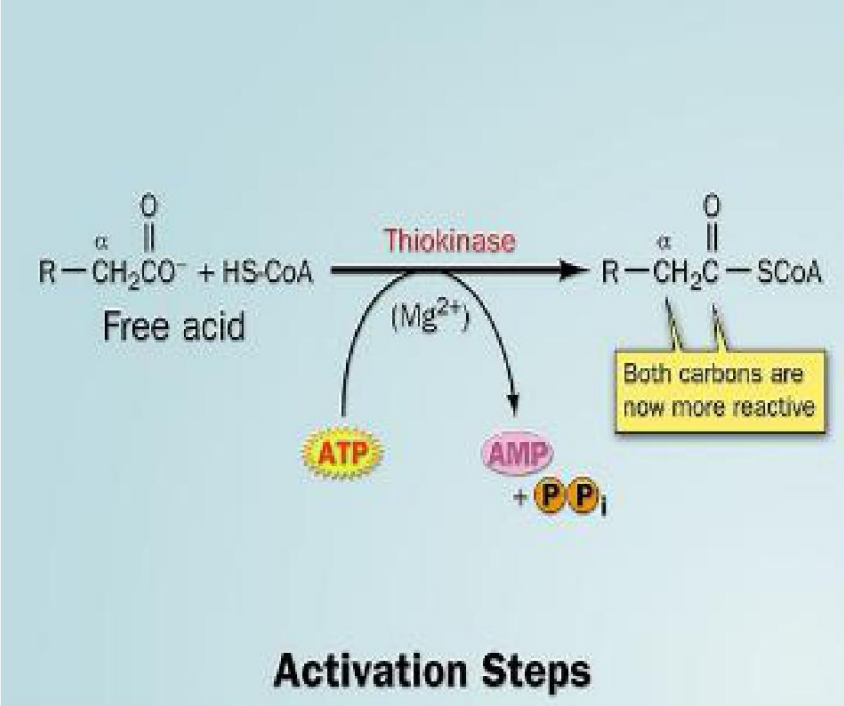
- Glucose entering the TCA cycle is used for the **biosynthesis of saturated fatty acids** by converting TCA Cycle citrate to acetyl coenzyme~A (acetyl~CoA), and then malonyl~CoA, which is used to produce palmitate.
- The glycerol backbone of TGs comes from glycolytic glycerol-3-phosphate.
- Triglycerides 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.

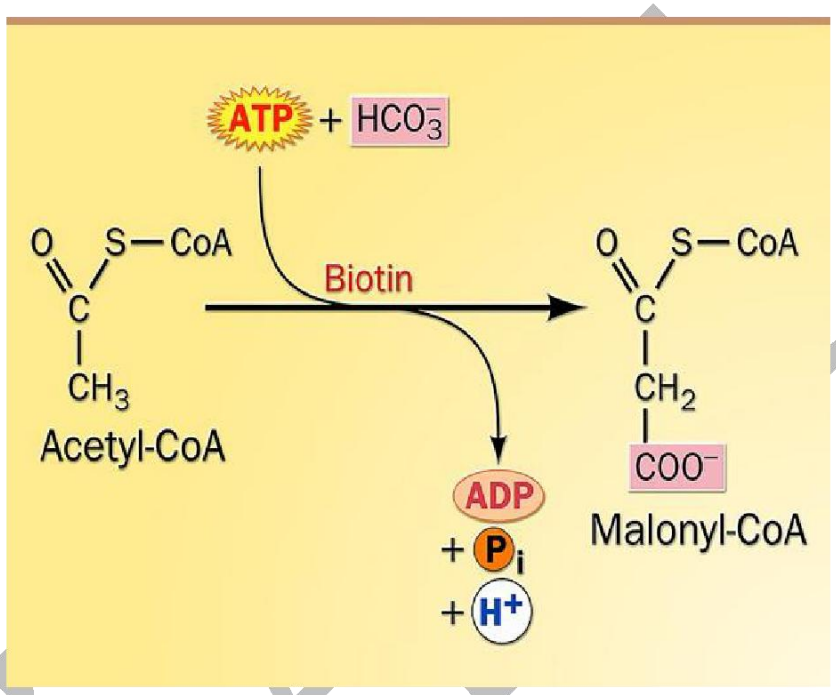


Fig: Acetyl coA to Malonyl coA

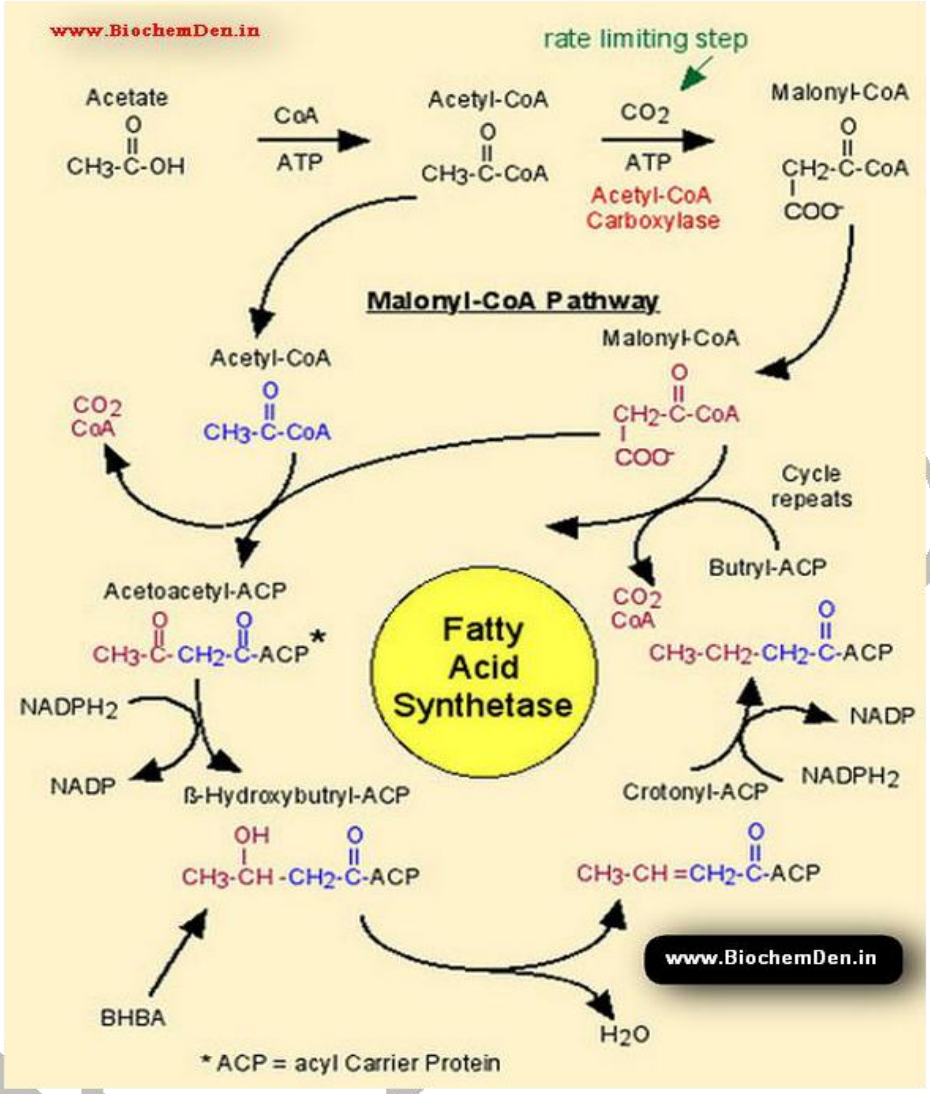
Fatty Acid Synthase Complex (FASC) Dimer:

- 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.

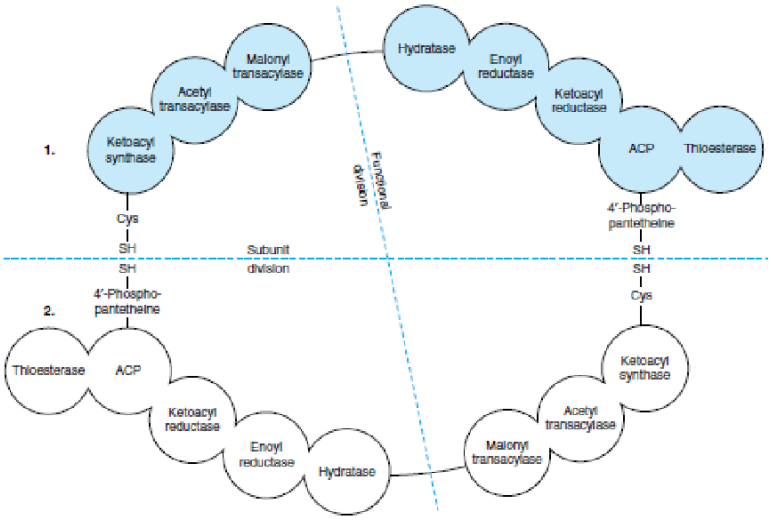
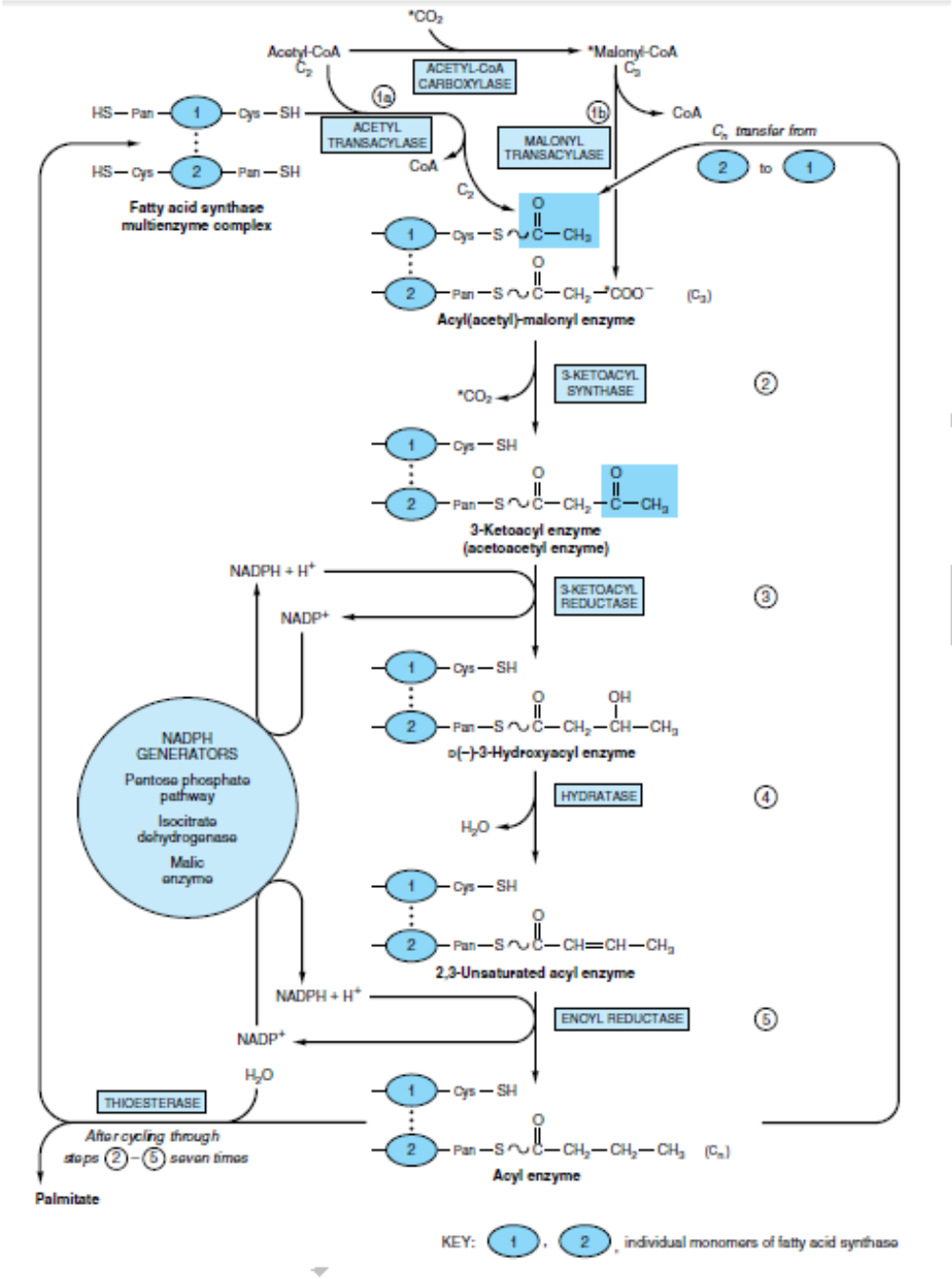


Fig: Fatty acid synthase complex

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.



Summary of fatty acid synthesis (ignoring H^+ and water):

acetyl-CoA + 7 malonyl-CoA + 14 NADPH → palmitate + 7 CO_2 + 14 $NADP^+$ + 8 CoA

Summary taking into account ATP-dependentsynthesis of malonate:

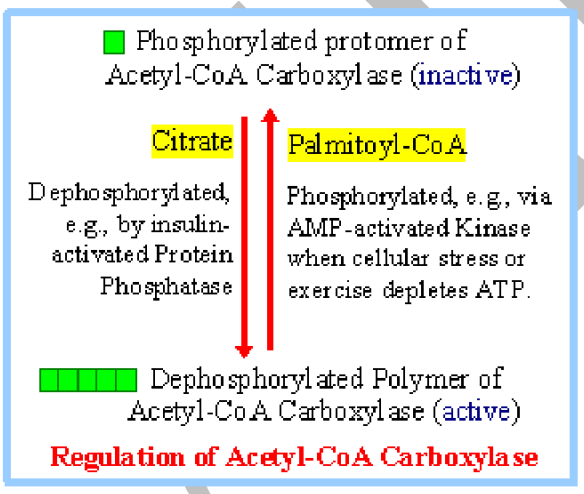
8 acetyl-CoA + 14 NADPH + 7 ATP → palmitate + 14 $NADP^+$ + 8 CoA + 7 ADP + 7 P_i

Regulation of Acetyl-CoA Carboxylase:

Acetyl-CoA Carboxylase, which converts acetyl-CoA to malonyl-CoA, is the **committed step** of the fatty acid synthesis pathway. The mammalian enzyme is regulated by**phosphorylation**, and there is **allosteric control** via local metabolites. Conformational changes associated with regulation:

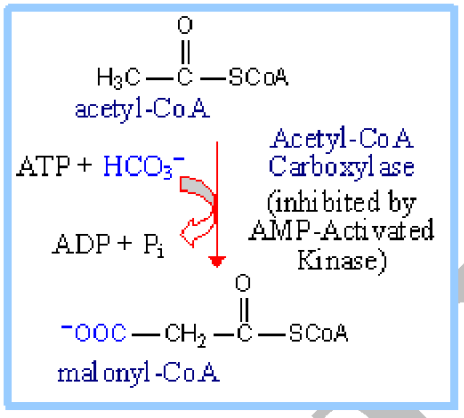
- When in the **active** conformation, Acetyl-CoA Carboxylase self-associates to form multimeric **filamentous** complexes. See electron micrograph p. 932 of Biochemistry, 3rd Edition, by Voet & Voet.

Transition to the **inactive** conformation is associated with dissociation to yield the **monomeric** form of the enzyme (protomer).



AMP functions as an energy sensor and regulator of metabolism. When ATP production does not keep up with needs, a higher portion of a cell's adenine nucleotide pool is in the form of AMP. AMP promotes catabolic pathways that lead to synthesis of ATP, while inhibiting energy-utilizing synthetic pathways. For example, AMP regulates fatty acid synthesis and catabolism by controlling availability of **malonyl-CoA**.

AMP-Activated Kinase catalyzes phosphorylation of **Acetyl-CoA Carboxylase** causing **inhibition** of the ATP-utilizing **production of malonyl-CoA** . Fatty acid synthesis is diminished by lack of the substrate malonyl-CoA. Fatty acid oxidation is stimulated due to decreased inhibition by malonyl-CoA of transfer of fatty acids into mitochondria



A cyclic-AMP cascade, activated by the hormones glucagon and epinephrine when blood glucose is low, may also result in phosphorylation of Acetyl-CoA Carboxylase via **cAMP-Dependent Protein Kinase**. With Acetyl-CoA Carboxylase inhibited, acetyl-CoA remains available for synthesis of ketone bodies, the alternative metabolic fuel used when blood glucose is low. The antagonistic effect of **insulin**, produced when blood glucose is high, is attributed to activation of Protein Phosphatase.

Regulation of Acetyl-CoA Carboxylase by local metabolites:

- **Palmitoyl-CoA**, the product of Fatty Acid Synthase, promotes the **inactive** conformation of Acetyl-CoA Carboxylase (diagram above), diminishing production of malonyl-CoA, the precursor of fatty acid synthesis. This is an example of feedback inhibition.
- **Citrate** allosterically **activates** Acetyl-CoA Carboxylase. Citrate concentration is high when there is adequate acetyl-CoA entering Krebs Cycle. Excess acetyl-CoA is then converted via malonyl-CoA to fatty acids for storage.

Fatty Acid Synthase is **transcriptionally regulated**.

- In **liver**:
    - Fatty Acid Synthase expression is **stimulated by insulin**, a hormone produced when blood glucose is high. Thus excess glucose is stored as fat. Transcription factors that that mediate the stimulatory effect of insulin include **USFs** (upstream stimulatory factors) and **SREBP-1**. SREBPs (sterol response element binding proteins) were first identified for their role in regulating cholesterol synthesis.

- Polyunsaturated **fatty acids diminish** transcription of the Fatty Acid Synthase gene in liver cells, by suppressing production of SREBPs.

- In **fat cells**: Expression of SREBP-1 and of Fatty Acid Synthase is **inhibited by leptin**, a hormone that has a role in regulating food intake and fat metabolism. Leptin is produced by fat cells in response to excess fat storage. Leptin regulates body weight by decreasing food intake, increasing energy expenditure, and inhibiting fatty acid synthesis.

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

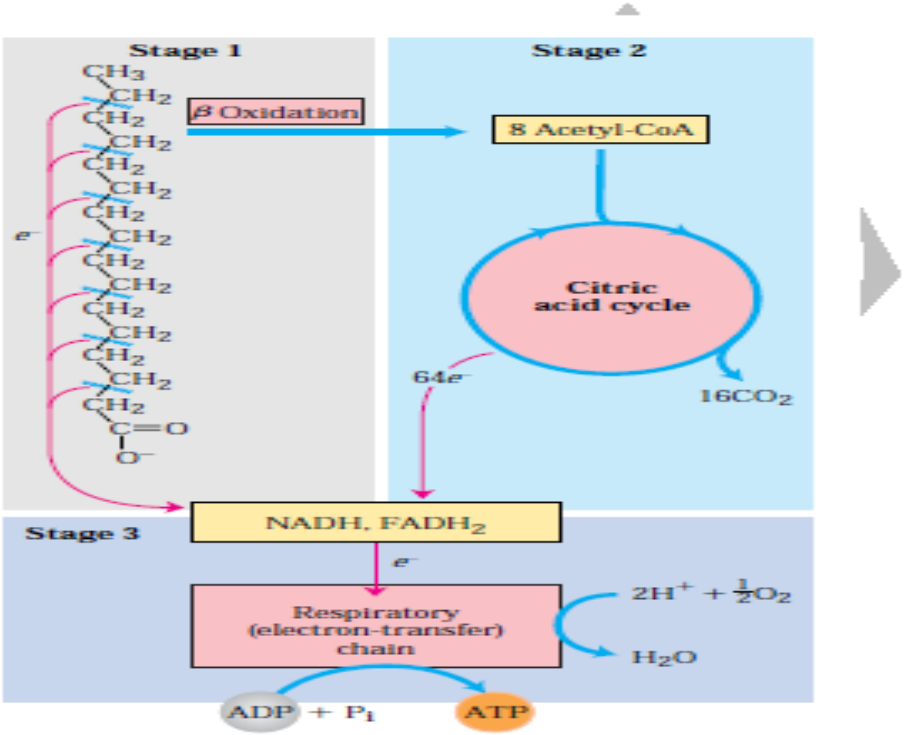
- Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression governing rates of synthesis of enzymes.

FATTY ACID OXIDATION

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.

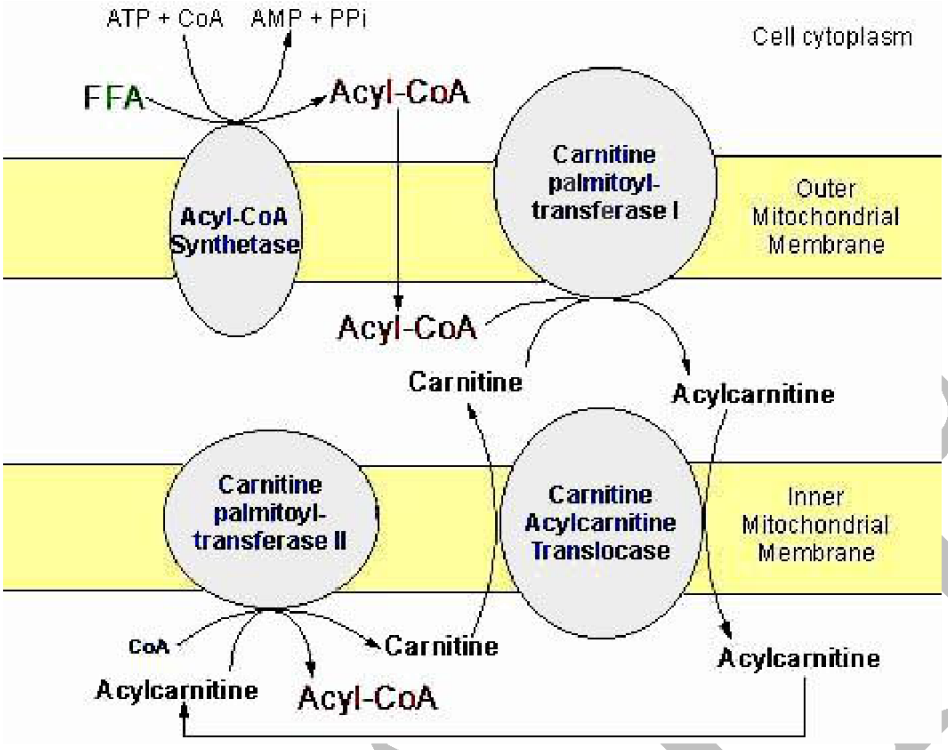
b) Trnasport of fattyacid into Mitochondria

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.

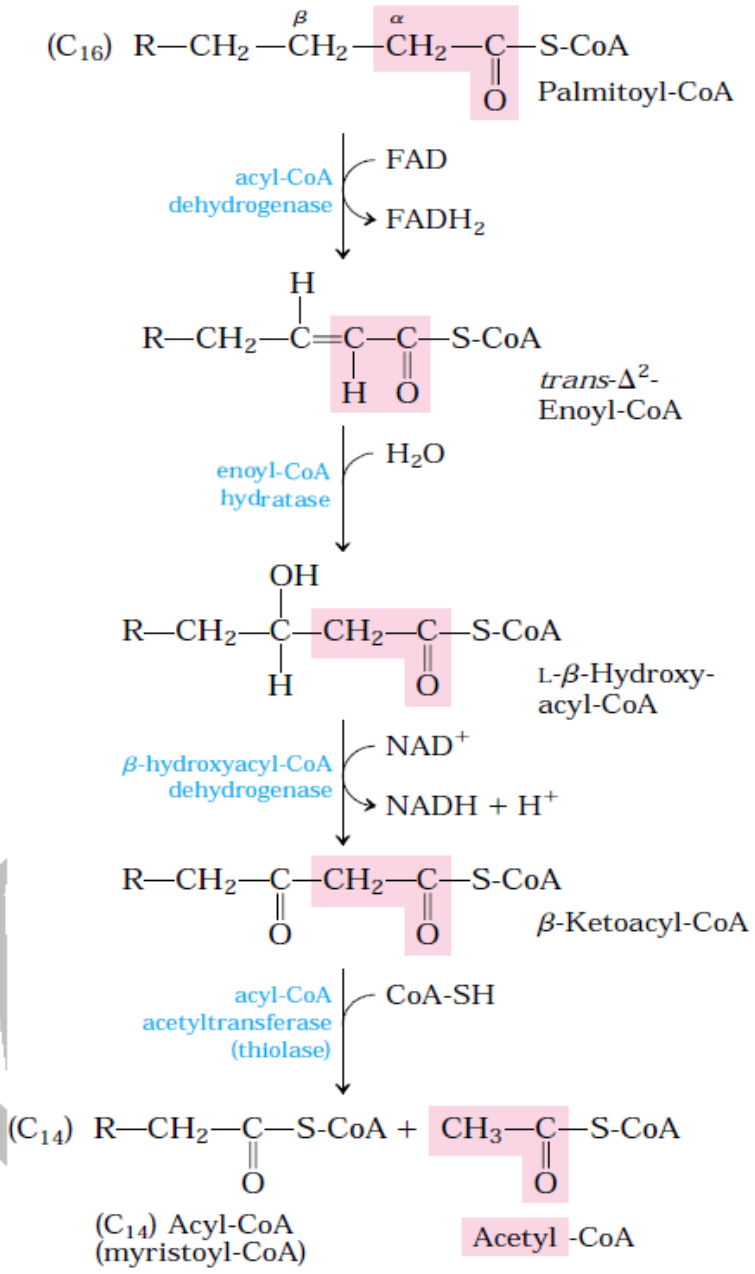
This first step is catalyzed by three isozymes of **acyl-CoA dehydrogenase,** each specific for a range of fatty-acyl chain lengths: very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons; medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons; and short-chain (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.

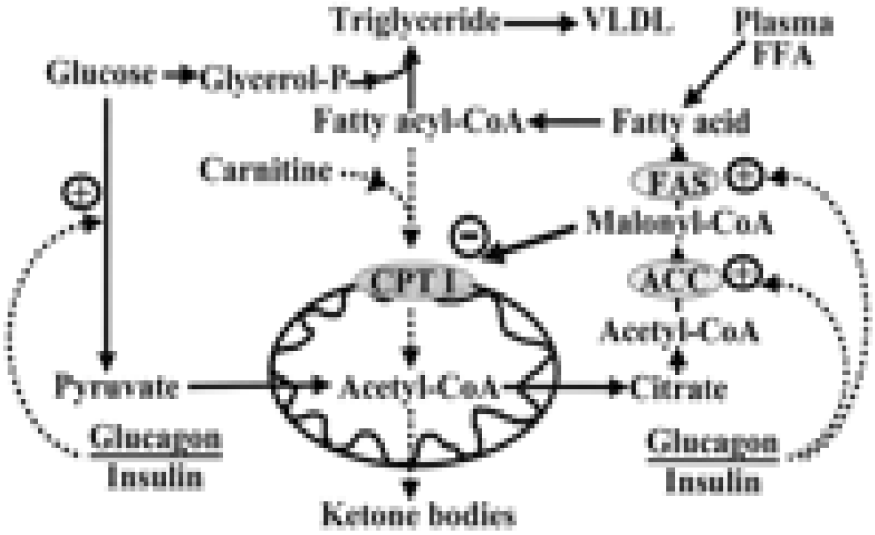
The β -oxidation sequence is an elegant mechanism for destabilizing and breaking these bonds. The first three reactions of β -oxidation create a much less stable C-C bond, in which the α_carbon (C-2) is bonded to *two* carbonyl carbons (the β -ketoacyl-CoA intermediate). The ketone function on the β- 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

RECIPROCAL CONTROL OF FATTYACID SYNTHESIS AND OXIDATION



Fatty acid synthesis and brta-oxidation pathways may be compared

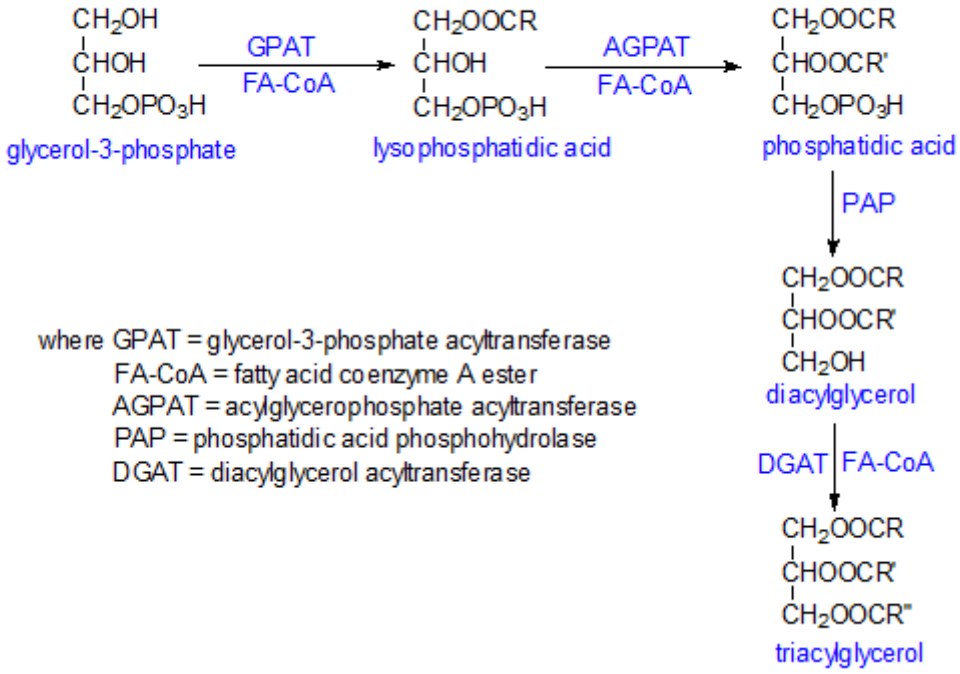
	Oxidation Pathway	Fatty Acid Synthesis
pathway location	mitochondrial matrix	cytosol
acyl carriers (thiols)	Coenzyme-A	phosphopantetheine (ACP) & cysteine
electron acceptors/donor	FAD & NAD^+	NADPH
hydroxyl intermediate	L	D
2-C product/donor	acetyl-CoA	malonyl-CoA (& acetyl-CoA)

Biosynthesis of Triacylglycerols

All eukaryotic organisms and even a few prokaryotes have the ability to synthesise triacylglycerols, and the process has been studied intensively in plants and animals especially. Many cell types and organs have the ability to synthesise triacylglycerols, but in animals the liver and intestines are most active, although most of the body stores of this lipid are in adipose tissue (see our web page on **triacylglycerol composition**). Within all cell types, even those of the brain, triacylglycerols are stored as cytoplasmic '**lipid droplets**' (also termed _fat globules‘, _oil

bodies‘, _lipid particles‘, _adiposomes‘, etc) enclosed by a monolayer of phospholipids and hydrophobic proteins, such as the perilipins in adipose tissue or oleosins in seeds. These lipid droplets are now treated as distinctive organelles, with their own characteristic metabolic pathways and associated enzymes – no longer boring blobs of fat. They are not unique to animals and plants as Mycobacteria and yeasts have similar lipid inclusions.

Two main biosynthetic pathways are known, the *sn*-glycerol-3-phosphate pathway, which predominates in liver and adipose tissue, and a monoacylglycerol pathway in the intestines. In maturing plant seeds and some animal tissues, a third pathway has been recognized in which a diacylglycerol transferase is involved. **Triacylglycerol biosynthesis in plants** is discussed in greater detail in a separate webpage on this site. The most important route to triacylglycerol biosynthesis is the *sn*-glycerol-3-phosphate or **Kennedy pathway** illustrated below, first described by Professor Eugene Kennedy and colleagues in the 1950s, by means of which more than 90% of liver triacylglycerols are produced.



In the *sn*-glycerol-3-phosphate or α-glycerophosphate pathway, the main source of the glycerol backbone has long been believed to be *sn*-glycerol-3-phosphate produced by the catabolism of glucose (glycolysis) or to a lesser extent by the action of the enzyme glycerol kinase on free

glycerol. However, there is increasing evidence that a significant proportion of the glycerol is produced *de novo* by a process known as glyceroneogenesis via pyruvate. Indeed, this may be the main source in adipose tissue.

Subsequent reactions occur in the endoplasmic reticulum. First, the precursor *sn*-glycerol-3-phosphate is esterified by a fatty acid coenzyme A ester in a reaction catalysed by a glycerol-3-phosphate acyltransferase (GPAT) at position *sn*-1 to form lysophosphatidic acid, and this is in turn acylated by an acylglycerophosphate acyltransferase in position *sn*-2 to form a key intermediate in the biosynthesis of all glycerolipids - **phosphatidic acid**.

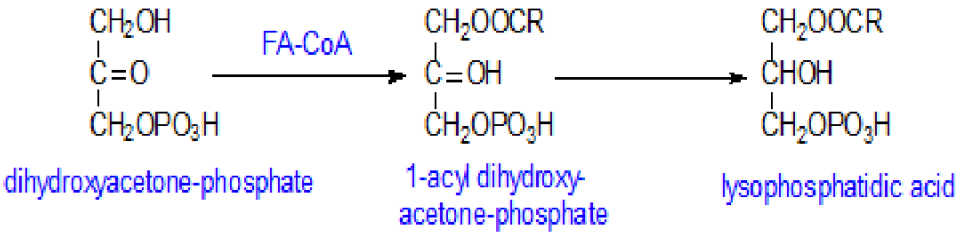
The phosphate group is removed by the enzyme phosphatidic acid phosphohydrolase (PAP or ‗phosphatidate phosphatase' or ‗lipid phosphate phosphatase'). PAP is also important as it produces diacylglycerols as essential intermediates in the biosynthesis of phosphatidylcholine and phosphatidylethanolamine. In contrast to the activity responsible for phospholipid biosynthesis in mammals, much of the phosphatase activity leading to triacylglycerol biosynthesis resides in three related cytoplasmic proteins, termed lipin-1, lipin-2, and lipin-3, which were characterised before the nature of their enzymatic activities were determined. The lipins are tissue specific, and each appears to have distinctive expression and functions, but lipin-1 (PAP1) accounts for all the PAP activity in adipose tissue and skeletal muscle. While it occurs mainly in the cytosolic compartment of cells, it is translocated to the endoplasmic reticulum in response to elevated levels of fatty acids within cells. Lipin-1 activity requires Mg^{2+} ions and is inhibited by *N*-ethylmaleimide, whereas the membrane-bound activity responsible for synthesising diacylglycerols as a phospholipid intermediate is independent of Mg^{2+} concentration and is not sensitive to the inhibitor.

Finally, the resultant 1,2-diacyl-*sn*-glycerol is acylated by a diacylglycerol acyltransferase (DGAT) to form the triacyl-*sn*-glycerol. As the glycerol-3-phosphate acyltransferase has the lowest specific activity of these enzymes, this step may be the rate-limiting one. On the other hand, DGAT is the dedicated triacylglycerol-forming enzyme, and this is seen as a target for pharmaceutical intervention in obesity and attendant ailments.

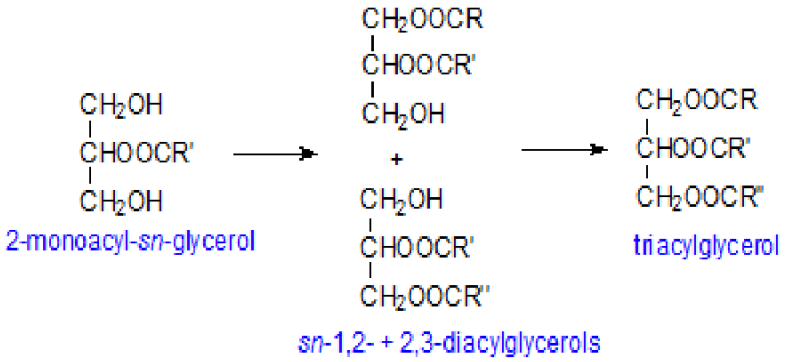
In fact there are two DGAT enzymes, which are structurally and functionally distinct. DGAT1 is expressed in skeletal muscle, skin and intestine, with lower levels of expression in

liver and adipose tissue. Perhaps surprisingly, it is the only one present in the epithelial cells that synthesise milk fat in the mammary gland. DGAT2 is the main form of the enzyme in hepatocytes and adipocytes, although it is expressed much more widely in tissues. Both enzymes are important modulators of energy metabolism, although DGAT2 appears to be especially important in controlling the homeostasis of triacylglycerols *in vivo*.

Among other potential routes to the various intermediates, lysophosphatidic acid and phosphatidic acid can be synthesised in mitochondria, but must then be transported to the endoplasmic reticulum before they enter the pathway for triacylglycerol production. 1,2-Diacyl-*sn*-glycerols are produced by the action of phospholipase C on phospholipids. In addition, dihydroxyacetone-phosphate in peroxisomes or endoplasmic reticulum can be acylated by a specific acyltransferase to form 1-acyl dihydroxyacetone-phosphate, which is reduced by dihydroxyacetone-phosphate oxido-reductase to lysophosphatidic acid (part of the biosynthetic route to **plasmalogens**), which can then enter the pathway to triacylglycerols.

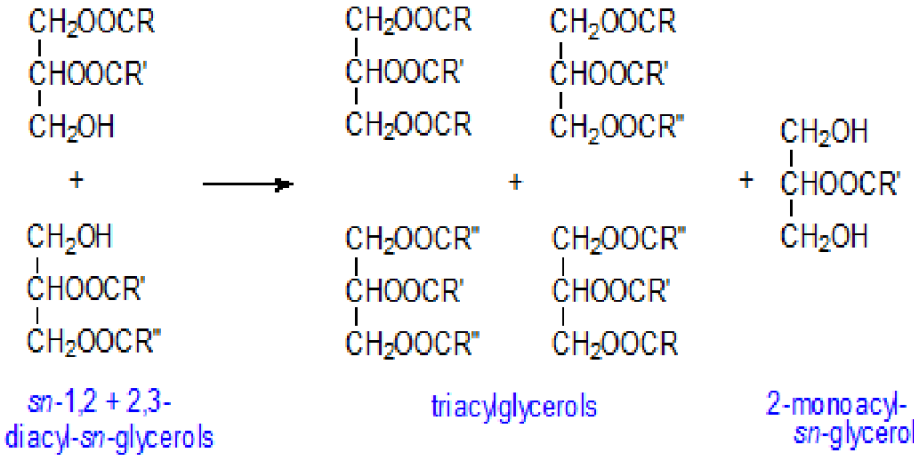


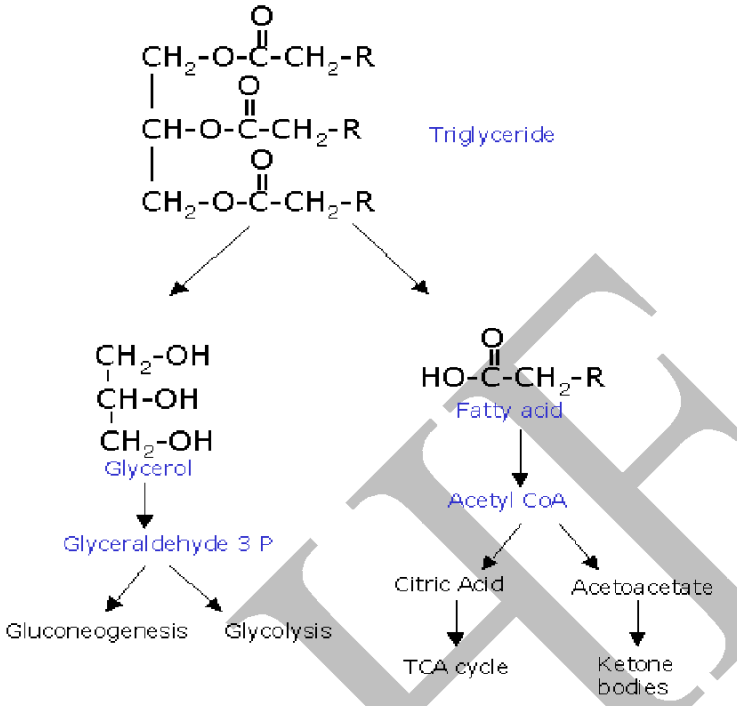
In the enterocytes of intestines after a meal, up to 75% of the triacylglycerols are formed via a monoacylglycerol pathway. 2-Monoacyl-*sn*-glycerols and free fatty acids released from dietary triacylglycerols by the action of pancreatic lipase within the intestines are taken up by the enterocytes. There, the monoacylglycerols are first acylated by an acyl coenzyme A:monoacylglycerol acyltransferase with formation of *sn*-1,2-diacylglycerols mainly as the first intermediate in the process, though *sn*-2,3-diacylglycerols are also produced, and then by acyl coenzyme A:diacylglycerol acyltransferase (DGAT1) to form triacylglycerols. DGAT1 can also acylate monoacylglycerols.



In the third biosynthetic pathway, which is less well known, triacylglycerols are synthesised by an acyl-CoA independent transacylation between two racemic diacylglycerols. The reaction was first detected in intestinal microvillus cells and is catalysed by a diacylglycerol transacylase. Both diacylglycerol enantiomers participate in the reaction with equal facility to transfer a fatty acyl group with formation of triacylglycerols and a **2-monoacyl-*sn*-glycerol**. A similar reaction has been observed in seed oils.

Triacyl glycerol catabolism





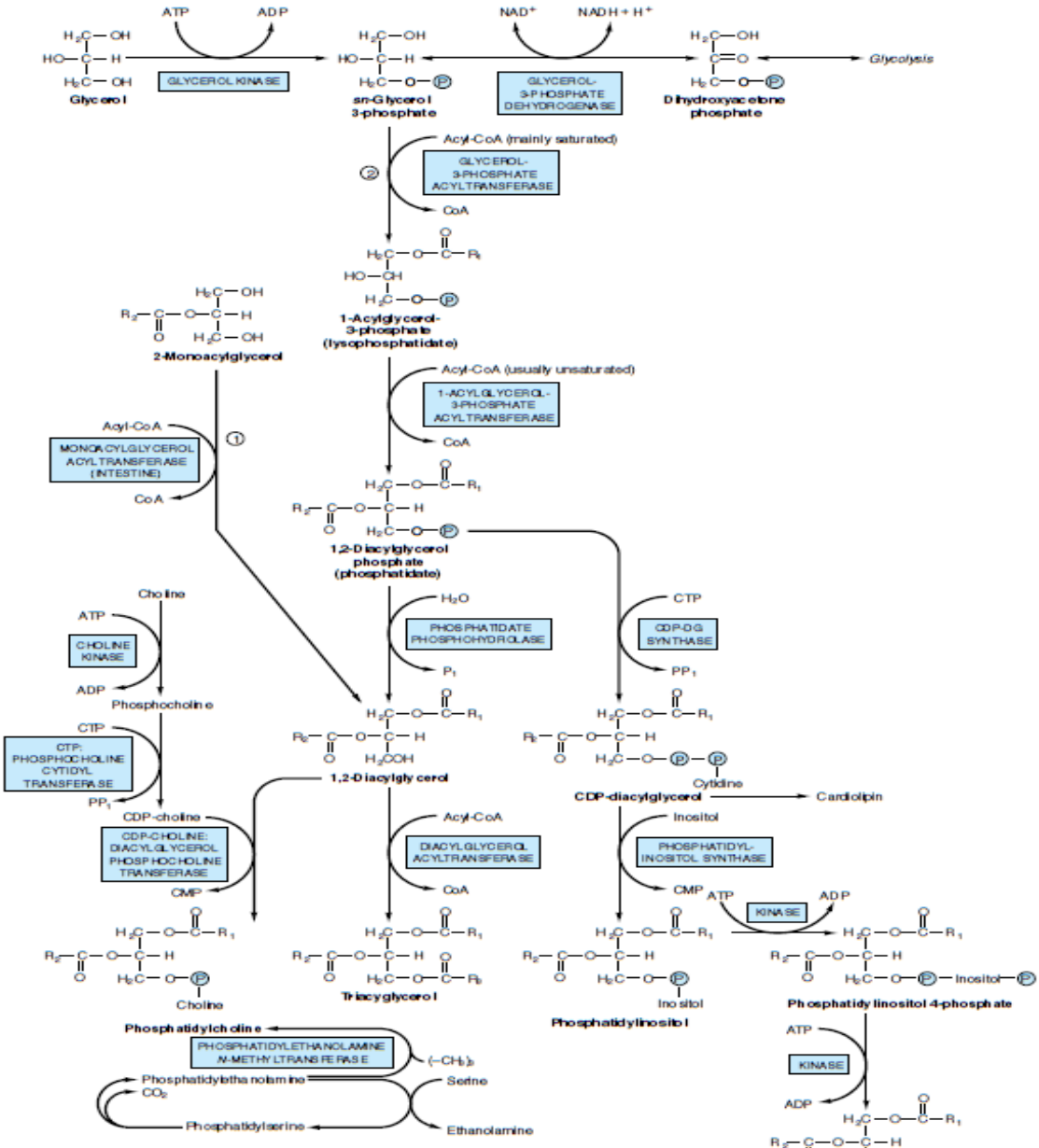
BIOSYNTHESIS OF PHOSPHOLIPIDS

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.

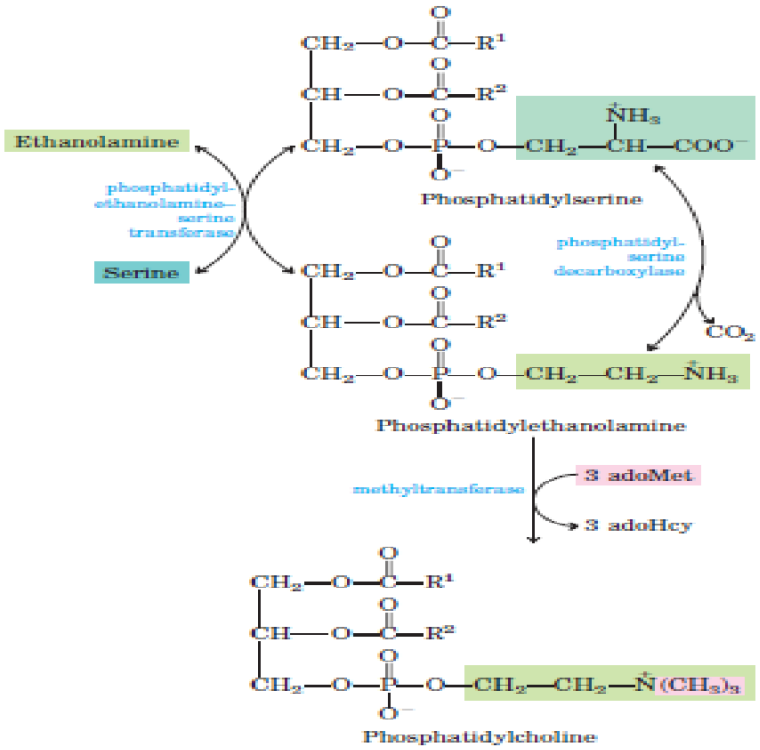


Biosynthesis of triacylglycerol and phospholipids. (①, Monoacylglycerol pathway; ②, glycerol phosphate pathway.) Phosphatidylethanolamine may be formed from ethanolamine by a pathway similar to that shown for the formation of phosphatidylcholine from choline.

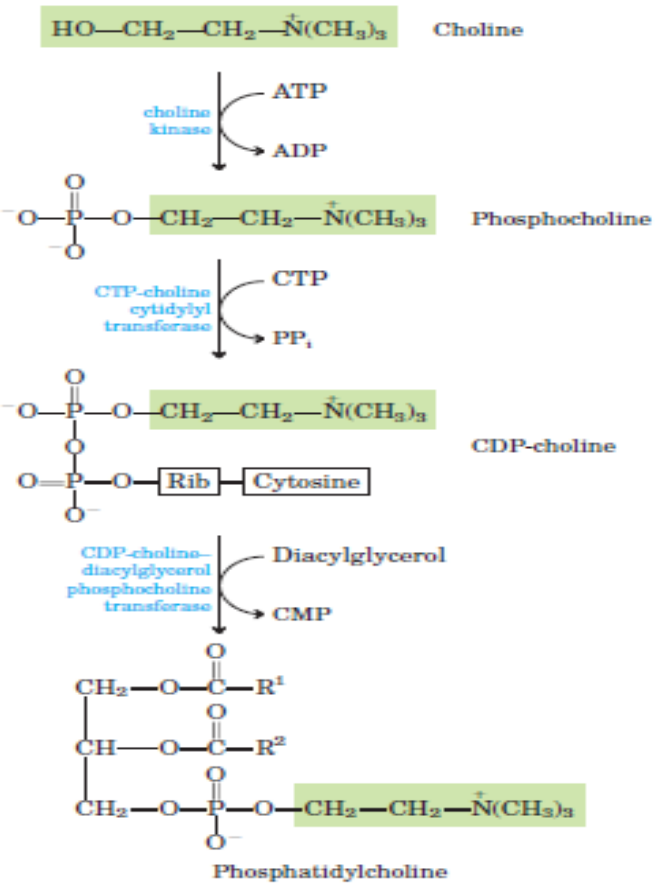
Specific **phosphatidylinositol kinases** then convert phosphatidylinositol to its phosphorylated derivatives. Phosphatidylinositol and its phosphorylated products in the plasma membrane play a central role in signal transduction in eukaryotes.

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.

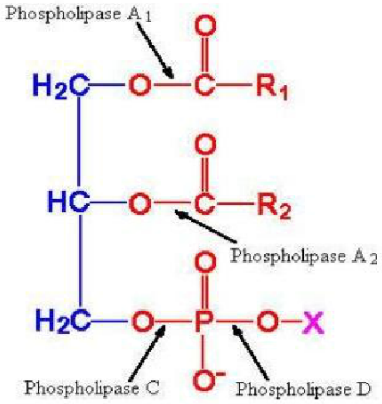
Phospholipase A2 hydrolyses the fatty acid at C_1 position of phospholipids. Snake venom and bee venom are rich sources of phospholipase A2. This enzyme is found in many tissues and pancreatic juice. Phospholipase A2 acts on phosphatidyl inositol to liberate arachidonic acid, the substrate for the synthesis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

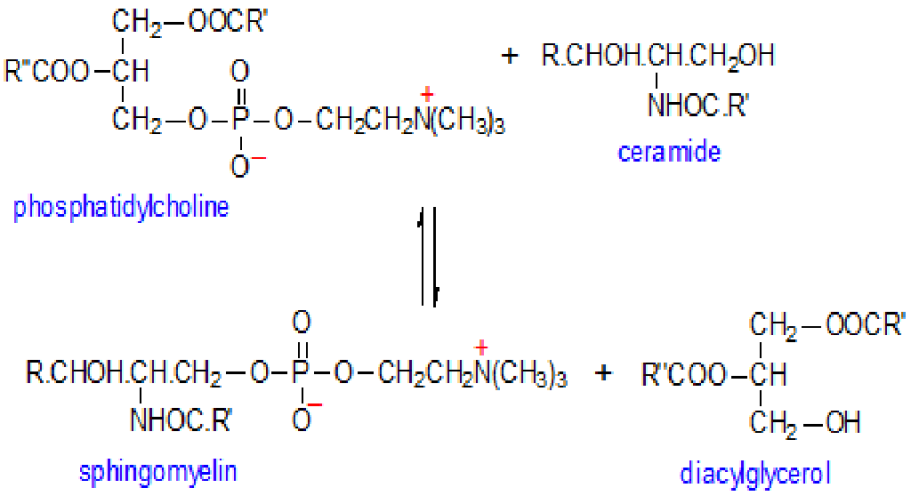
Phospholipid catabolism

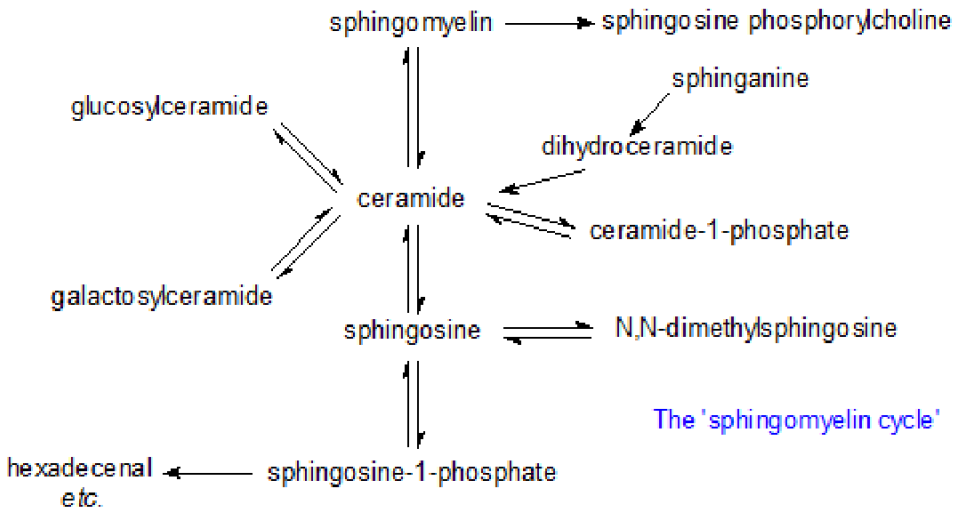
Degradation of phospholipids



Biosynthesis of Sphingomyelin

The biosynthesis of sphingomyelin is distinct from that of **phosphatidylcholine**. Indeed, it involves transfer of phosphorylcholine from phosphatidylcholine to ceramide, liberating diacylglycerols, and catalysed by a ceramide choline-phosphotransferase (sphingomyelin synthase). The reaction takes place in the plasma membrane and Golgi, with distinct integral enzymes in each organelle. A specific ceramide transport molecule (CERT) is important to the reaction (see our web page on **ceramides**) in that it transfers ceramide from the endoplasmic reticulum to the Golgi in a non-vesicular manner.





Sphingomyelin Catabolism

The key enzymes for the degradation of sphingomyelin to ceramides in most tissues are sphingomyelinases, which are similar in function to phospholipase C. There are a number of such enzymes with differing pH optima that appear to operate in different regions of the cell with potentially distinct biochemical roles. For example, there is an acid sphingomyelinase in the endo-lysosomes, and different neutral sphingomyelinases in the plasma membrane, endoplasmic reticulum, Golgi and mitochondria. A diverse range of factors activate the enzymes, including chemotherapeutic agents, tumor necrosis factor-alpha, 1,25-dihydroxy-vitamin D_3, endotoxin, gamma-interferon, interleukins, nerve growth factor, and most conditions known to induce cellular stress. In that they generate ceramides and other sphingolipid metabolites that have important signalling functions, sphingomyelinases are believed to function as regulators of signalling mechanisms, especially in the nucleus of the cell. The lysosomal acid sphingomyelinase may be involved in recycling of sphingolipid constituents.

Cholesterol Synthesis

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.

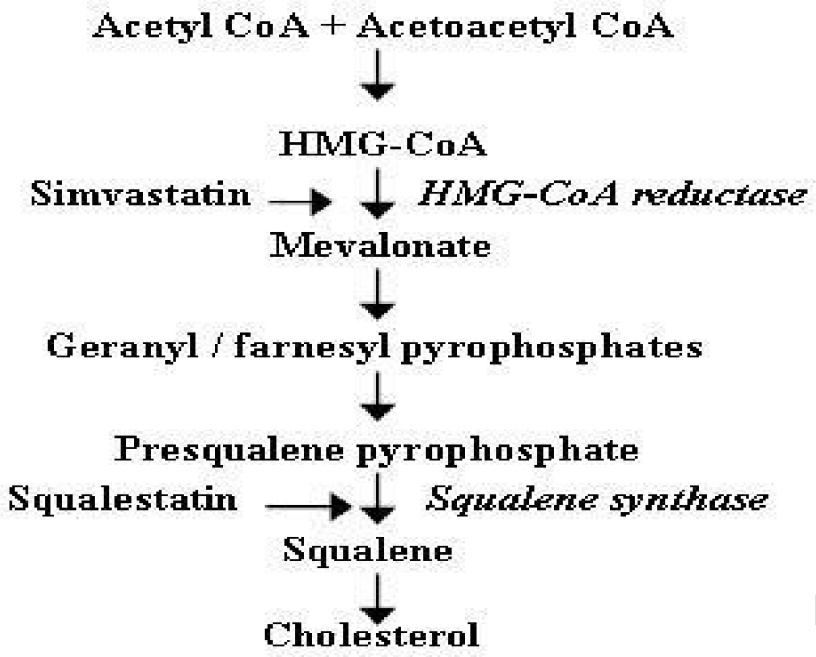
Steps:

1.HMG-CoA formation and conversion to mevalonate

2.Conversion of mevalonate to isoprenoid precursors

3.Synthesis of squalene and its conversion to lanosterol

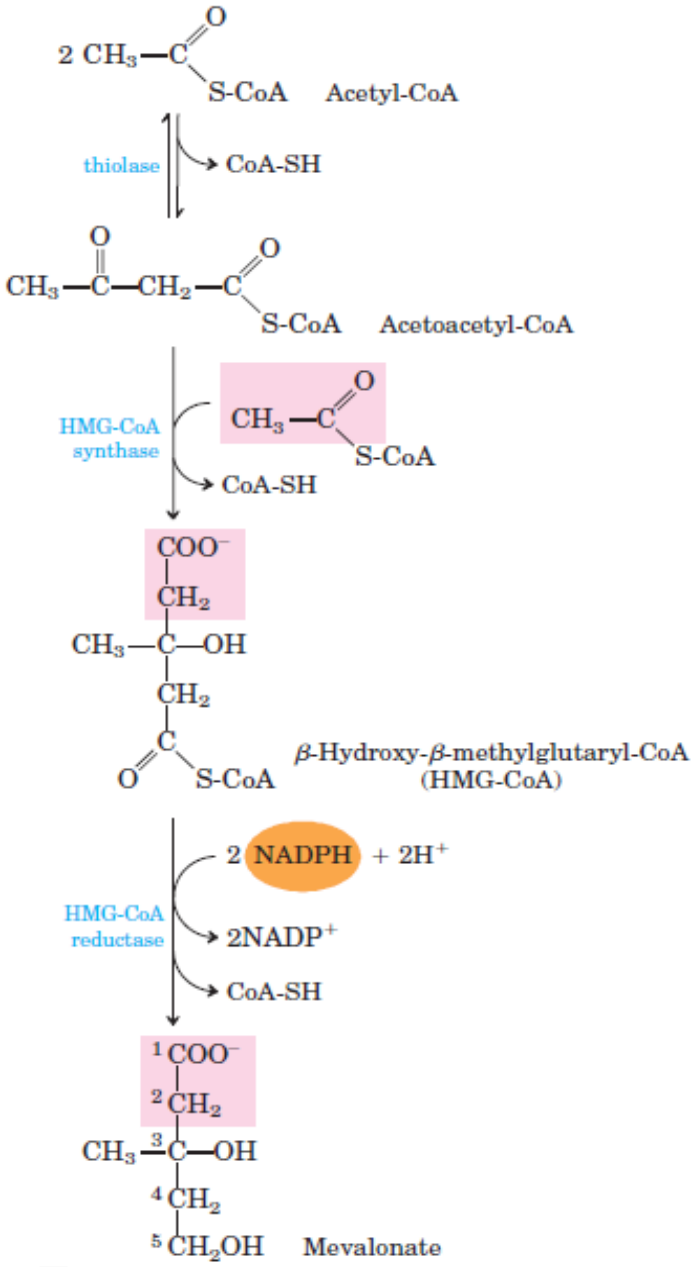
4. Conversion of lanosterol to cholesterol



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.

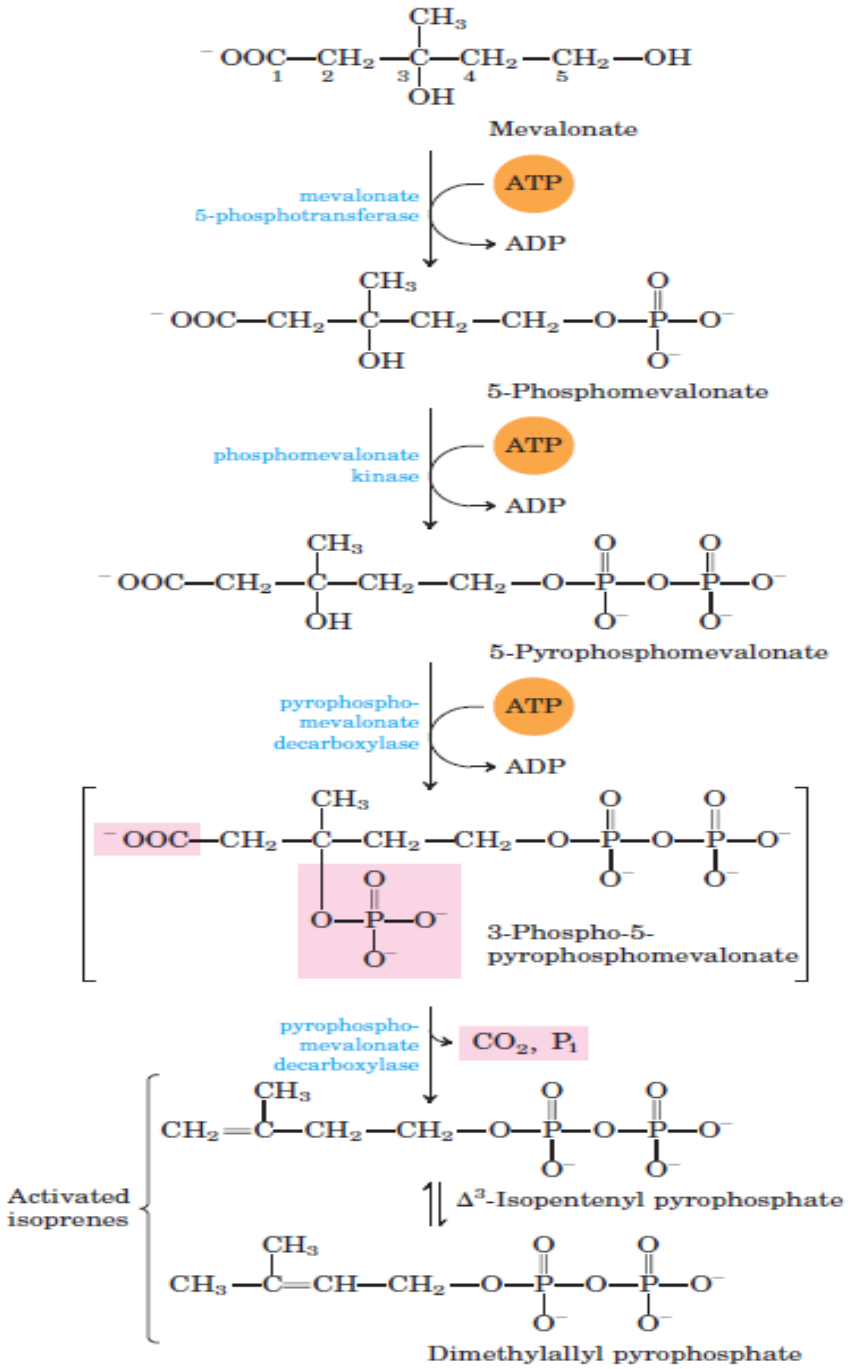
Formation of mevalonate from acetyl-CoA: The origin of C-1 and C-2 of mevalonate from acetyl-CoA is shown in pink.



Stage 2 Conversion of Mevalonate to Two Activated Isoprenes

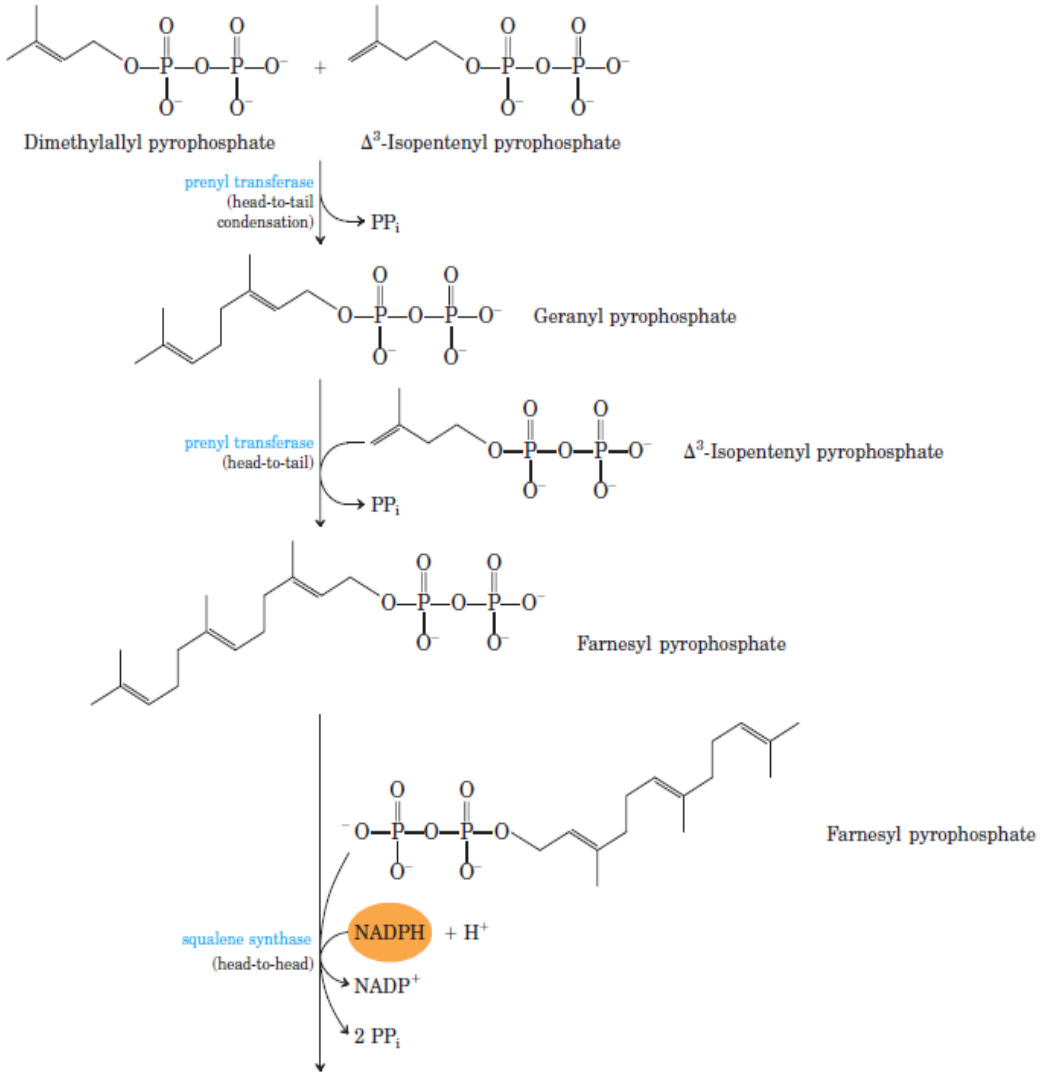
In the next stage of cholesterol synthesis, three phosphate groups are transferred from three ATP molecules to mevalonate. The phosphate attached to the C-3 hydroxyl group of mevalonate in the intermediate 3-phospho-5-pyrophosphomevalonate is a good leaving group; in the next step, both this phosphate and the nearby carboxyl group leave, producing a double bond

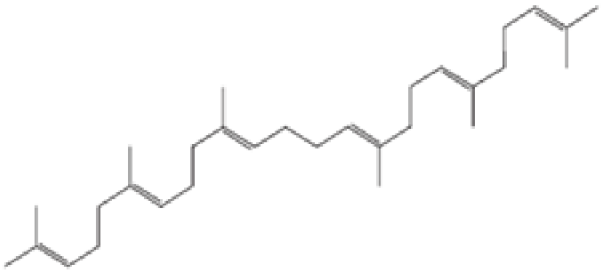
in the five-carbon product, Δ^3**-isopentenyl pyrophosphate.** This is the first of the two activated isoprenes central to cholesterol formation. Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, **dimethylallyl pyrophosphate.**



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.**

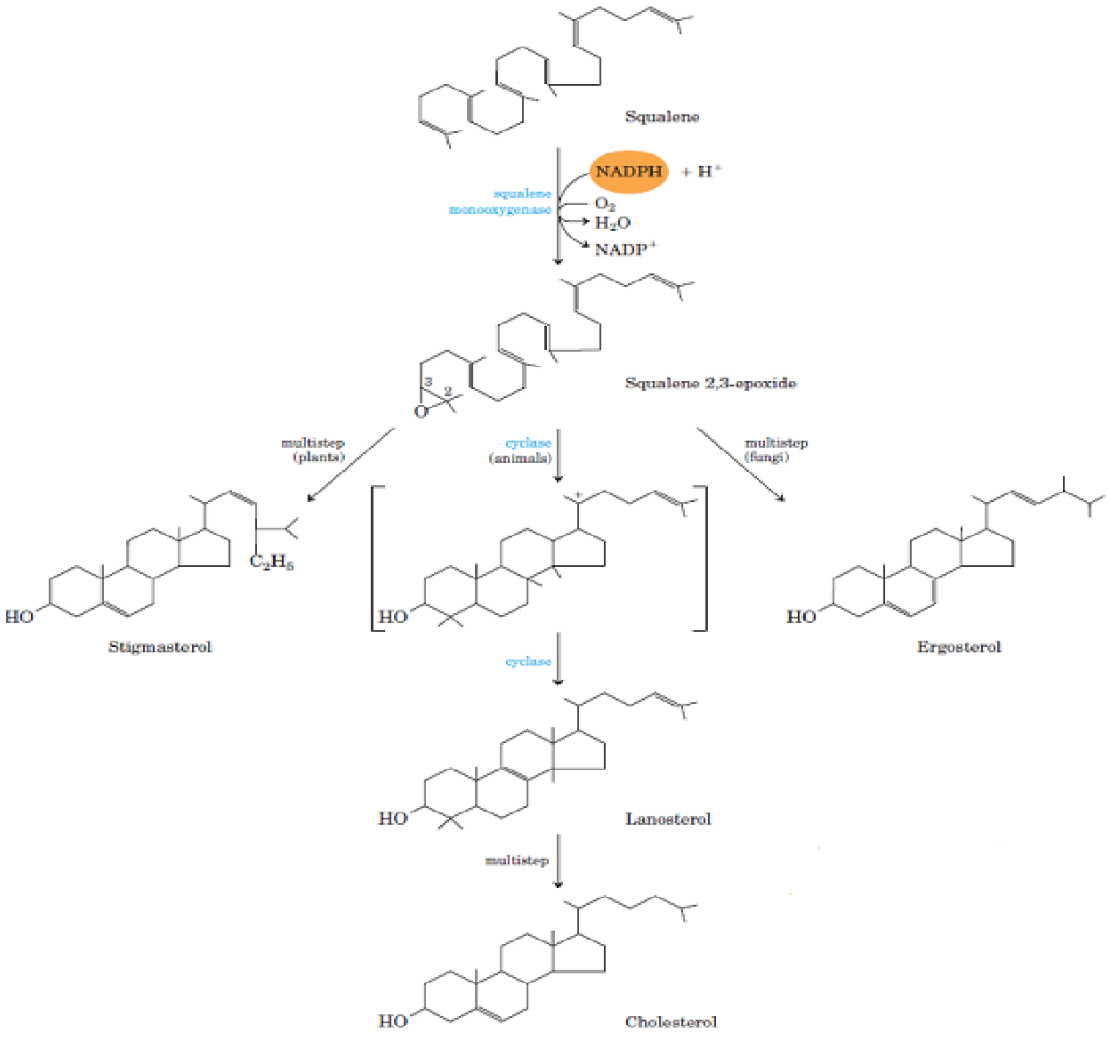




Squalene

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.

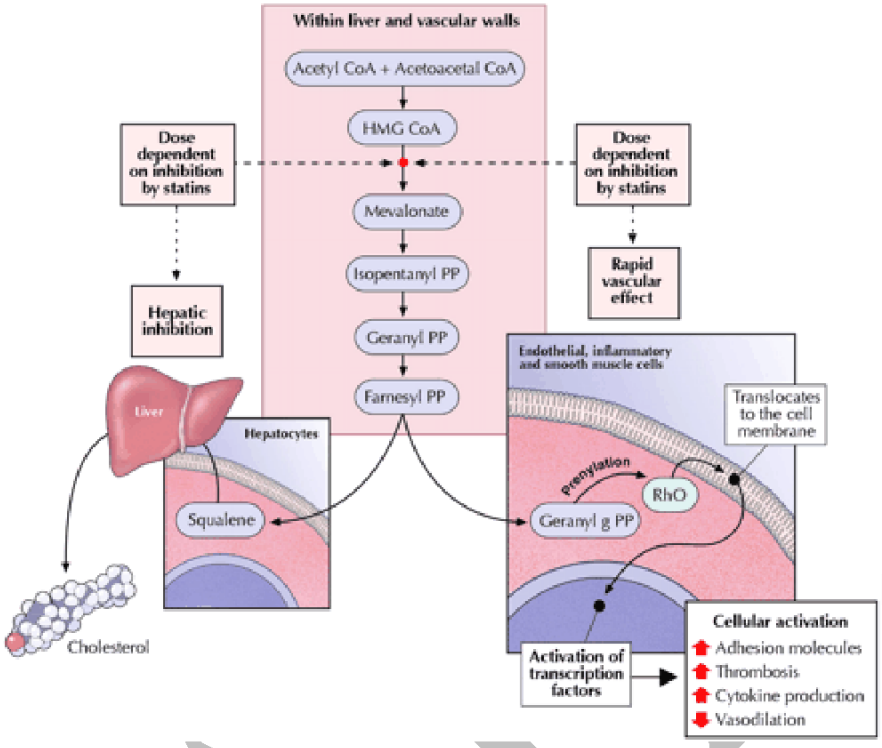
In the second stage of fatty acid oxidation, the acetyl-CoA is oxidized to CO_2 in the citric acid cycle. A large fraction of the theoretical yield of free energy from fatty acid oxidation is recovered as ATP by oxidative phosphorylation, the final stage of the oxidative pathway. Malonyl-CoA, an early 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.

Regulation Of Cholesterol Syntheis

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

Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibits cholesterol synthesis and isoprenoid production (geranyl-geranyl pyrophosphate and farnesyl pyrophosphate). This in turn reduces prenylation of G proteins such as Rho, and hence membrane binding.



Regulation of HMG-CoA Reductase

HMG-CoA Reductase, the rate-determining step on the pathway for synthesis of cholesterol, is a major control point. Regulation relating to cellular uptake of cholesterol will be discussed in the next class.

Short-term regulation

- HMG-CoA Reductase is inhibited by **phosphorylation**, catalyzed by **AMP-Dependent Protein Kinase** (which also regulates fatty acid synthesis and catabolism). This kinase is active when cellular AMP is high, corresponding to when ATP is low. Thus, when cellular ATP is low, energy is not expended in synthesizing cholesterol.

Long-term regulation of cholesterol synthesis is by varied **formation** and **degradation** of HMG-CoA Reductase and other enzymes of the pathway for synthesis of cholesterol.

- **Regulated proteolysis** of **HMG-CoA Reductase**: Degradation of HMG-CoA Reductase is stimulated by cholesterol, by oxidized derivatives of cholesterol, by mevalonate, and by farnesol (dephosphorylated farnesyl pyrophosphate). HMG-CoA Reductase includes a transmembrane **sterol-sensing domain** that has a role in activating degradation of the

enzyme via the proteasome. (The proteasome is discussed separately in the section on protein degradation.)

- **Regulated transcription**: A family of transcription factors designated **SREBP** (sterol regulatory element binding proteins) regulate synthesis of cholesterol and fatty acids. Of

these, **SREBP-2** mainly **regulates cholesterol synthesis**. (SREBP-1c mainly regulates fatty acid synthesis.)

When sterol levels are low, SREBP-2 is released by cleavage of a membrane-bound precursor protein. SREBP-2 **activates transcription** of genes for **HMG-CoA Reductase** and other enzymes of the pathway for cholesterol synthesis.

Atherosclerosis

Atherosclerosis (also known as **arteriosclerotic vascular disease** or **ASVD**) is a condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL), (seeapoA-1 Milano). It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries. The atheromatous plaque is divided into three distinct components:

1. The atheroma ("lump of gruel," from ἀθήρα, *athera*, gruel in Greek), which is the nodular accumulation of a soft, flaky, yellowish material at the center of large plaques, composed of macrophages nearest the lumen of the artery
2. Underlying areas of cholesterol crystals
3. Calcification at the outer base of older/more advanced lesions.

These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures (seevulnerable plaque), causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes. This catastrophic event is called an infarction. One of the most common recognized scenarios is called coronary thrombosis of a coronary artery,

causing myocardial infarction (a heart attack). The same process in an artery to the brain is commonly called stroke. Another common scenario in very advanced disease is claudication from insufficient blood supply to the legs, typically caused by a combination of both stenosis and aneurysmal segments narrowed with clots. Since atherosclerosis is a body-wide process, similar events occur also in the arteries to the brain, intestines, kidneys, legs, etc. Many infarctions involve only very small amounts of tissue and are termed clinically silent, because the person having the infarction does not notice the problem, does not seek medical help or when they do, physicians do not recognize what has happened.

Signs and symptoms

Atherosclerosis typically begins in early adolescence, and is usually found in most major arteries, yet is asymptomatic and not detected by most diagnostic methods during life. Atheroma in arm, or more often in leg arteries, which produces decreased blood flow is called peripheral artery occlusive disease (PAOD).

Causes

Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized (ldl-ox) by free radicals[*citation needed*], particularly reactive oxygen species (ROS). When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL.

Risk factors

Various anatomic, physiological and behavioral risk factors for atherosclerosis are known. These can be divided into various categories: congenital *vs* acquired, modifiable or not, classical or non-classical. The points labelled '+' in the following list form the core components of metabolic syndrome. Risks multiply, with two factors increasing the risk of atherosclerosis fourfold. Hyperlipidemia, hypertension and cigarette smoking together increases the risk seven times.

Hyperlipoproteinemia

H**yperlipoproteinemia** is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma.

\ Hyperlipidemia (ie, elevated plasma cholesterol or triglyceride levels or both) is present in all hyperlipoproteinemias. The primary form includes chylomicronemia, hypercholesterolemia, dysbetalipoproteinemia,hypertriglyceridemia, mixed hyperlipoproteinemia, and combined hyperlipoproteinemia. Other diseases, such as diabetes mellitus,pancreatitis, renal disease, and hypothyroidism, cause the secondary form.

Combined hyperlipidemia a generic designation for a hyperlipidemia in which several classes of lipids are elevated; usually used to denote the phenotype of a type II-b hyperlipoproteinemia.

Familial combined hyperlipidemia an inherited disorder of lipoprotein metabolism manifested in adulthood as hypercholesterolemia, hypertriglyceridemia, or a combination, with elevated plasma apolipoprotein B and premature coronary atherosclerosis.

Remnant hyperlipidemia a form in which the accumulated lipoproteins are normally transient intermediates, chylomicron remnants, and intermediate-density lipoproteins; a generic descriptor for the type III hyperlipoproteinemia phenotype.

Hypolipoproteinemia

Hypolipoproteinemia **is defined as a lack of lipoprotein in the blood due to genetic or other diseases such as malnutrition and malabsorption**

Diagnosis

It can be diagnosed via blood study that identifies fat particles. The patient must fast overnight to prevent interference from fat in the blood due to food intake.

Treatment

Vitamin E supplements have shown to help children with the deficiency.

POSSIBLE QUESTIONS

2 mark questions

1. Enumerate the ATP produced by the oxidation of palmatic acid
2. How Acetyl CoA carboxylase is regulated
3. Add notes on Nieman pick disease
4. Explain with neat diagram about the role of carnitine cycle
5. In brief explain the synthesis of triacyl glycerol
6. 8) Draw the energetics of oxidation of palmitic acid
7. Explain the synthesis of phosphatidylethanolamine
8. Add notes on atheroclerosis
9. Sketch the fatty acid synthase complex
10. Brief out the synthesis of phosphatidylcholine
11. Sketch the regulation of triacyglecerol.

Essay type questions (6Marks)

1. Discuss the fatty acid oxidation and its regulation
2. Explain the synthesis and regulation of cholesterol
3. Narrate the fatty acid synthesis and its regulation
4. Synthesis and control of triacyl glycrol
5. Control of fattyacid synthesis by ACC and fatty acid synthase complex
6. Describe synthesis and regulation of phospholipids
7. Discuss the clinical conditions like atherosclerosis and hypo and hyper lipoproteinemia

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I MSc BIOCHEMISTRY-Second Semester

REGULATION OF METABOLIC PATHWAYS (18BCP201)

UNIT-III **MULTIPLE CHOICE QUESTIONS**

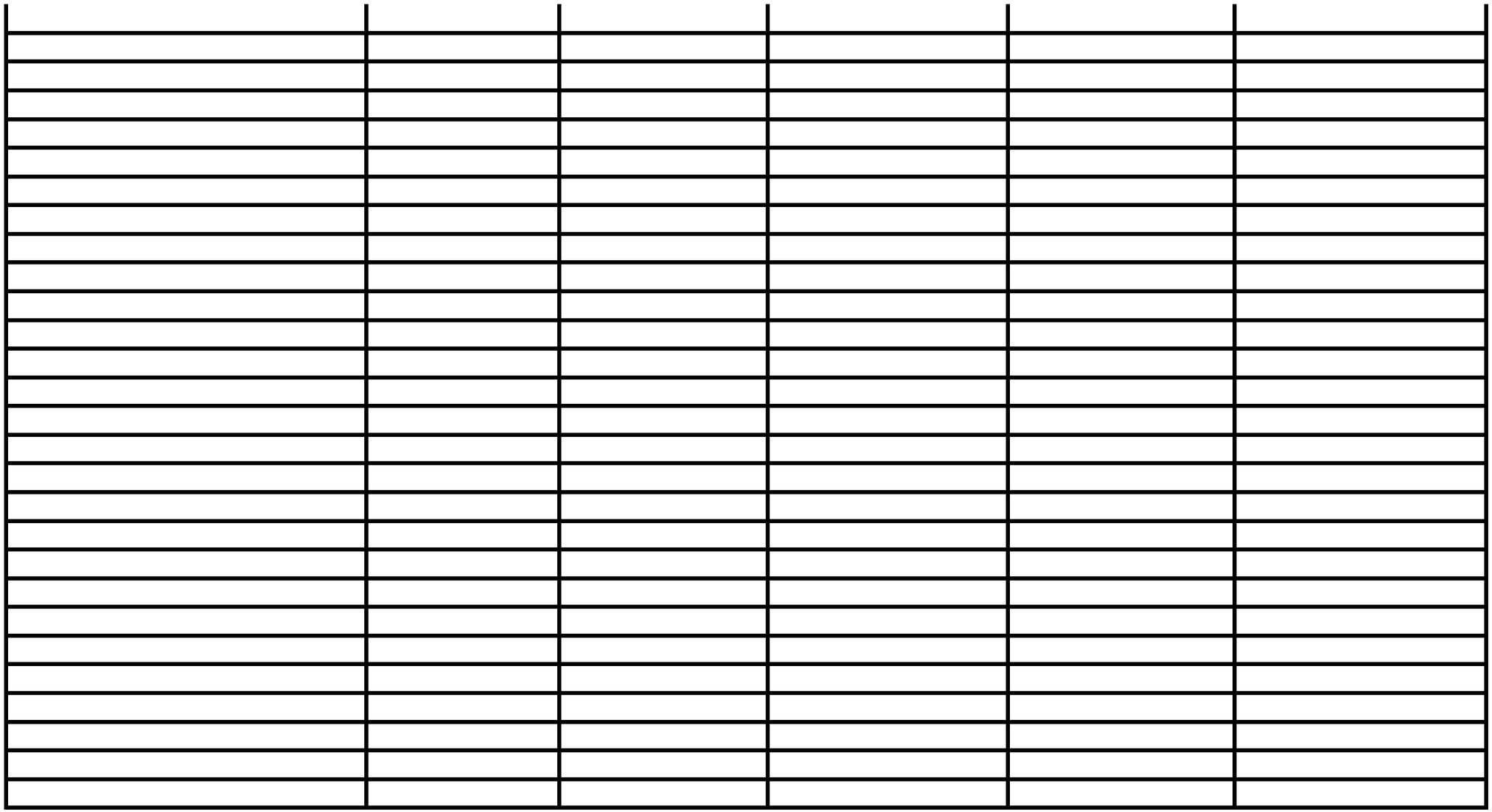
S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Cholesterol esters are comprised of cholesterol and _____	glycerol	a fatty acid	sphingosine	ceribroside	a fatty acid
2	Transporter of acyl molecules of long chain fatty acids _____	citrate	oxaloacetate	phosphoenol pyruvate	carnitine	carnitine
3	Adipose tissue requires glucose for the synthesis of triacyl glycerols because ____	acetyl CoA from glucose is converted into fatty acids	glucose is the precursor of glycerol -3-phosphate	the hormone sensitive lipase woul;d be phosphorylated and active	lactate can be converted into glycerol-3-phosphate	glucose is the precursor of glycerol -3-phosphate
4	Acetyl CoA carboxylase activity is enhanced by the presence of _____	low concentration of citrate	high concentration of citrate	low concentration of malonyl CoA	high concentration of malonyl CoA	high concentration of citrate
5	Fatty acid synthesis takes place in the presence of the co enzymes _____	NAD+	reduced NAD	reduced NADP	NADP+	reduced NADP
6	Chylomicron and VLDL are released from the intestine or the hepatic cell by _____	pinocytosis	diffusion	reverse osmosis	passive diffusion	reverse osmosis
7	Carboxylation of acetyl CoA to malonyl CoA takes place in presence of _____	FAD+	biotin	NAD+	NADP+	biotin
8	HDL is synthesized and secreted from _____-	pancrease	kidney	liver	muscle	liver
9	Enzyme for the fatty acid synthesis is located in _____	Cytoplasm	nucleus	mitochondria	peroxysones	Cytoplasm

10	Cholestrol synthesis is controlled by _____-	HMG CoA synthase	HMG CoA reductase	acetyl CoA carboxylase	acetyl CoA synthase	HMG CoA reductase
11	The basic structure of the sphingomyelin is _____-	cerebroside	ganglioside	sphingoshine	cholesterol	sphingoshine
12	High concentration of polyunsaturated fattyacid are present in _____	bread	vegetable oil	butter	milk	vegetable oil
13	_____ is the common precursor in the bio synthesis of the triacylglycerols many phosphoglycerols and cardiolipin.	phosphatidate	dihydroxy acetone phosphate	1,3,- diacylglycerol phosphate	1,2 –diacyl glycerol	phosphatidate
14	Which one of the following is the principal building blocks of fatty acids?	acetylacylCoA	acetylCoA	malonylCoA	3-ketoacylCoA	acetylCoA
15	Fatty acid synthesis is carried out at_____	Cytoplasm	nucleus	mitochondria	peroxysones	Cytoplasm
16	Fatty acid synthase is depressed by _____.	NAD+	reduced NAD	reduced NADP	NADH	reduced NADP
17	High intake of cholesterol diet decreases the activity of _____-	HMG CoA reductase	mevalonate kinase	HMG CoA synthetase	ATP citrate lyase	HMG CoA reductase
18	Lipo protein lipase is released by _____-	ephinephrine	sodium citrate	insulin	heparin	sodium citrate
19	_____ catalyses the formation of leucotrienes from arachidonate	prostoglandin synthase	prostocyclin synthase	lipoxygenase	cycloxygenase	lipoxygenase
20	Mammals cells lack the enzymes to introduce double bonds beyond carbon atom _____ in the fatty acid chain.	C5	C7	C9	C17	C9
21	The leucotrienes are a family of conjucated trienes formed from _____	eicosonoic acid	pentanoic acid	capric acid	cervonic acid	eicosonoic acid

22	Synthesis of mevolonate occurs from _____	lanosterol	acetyl CoA	squalene	HMG-CoA	acetyl CoA
23	Acetyl CoA carboxylase enzyme is activated by _____	citrate	glucagons	ephinephrine	palmitylCoA	citrate
24	Acetyl CoA carboxylase has an important requirement of vitamin _____	vitamin A	vit B12	biotin	ascorbic acid	biotin
25	The enzyme which converts long chain acyl CoA into acylcarnitine_____	carnitine palmityl transferase (I).	carnitine palmityl transferase (II)	carnitine acetyl transferase	acyl CoAsynthetase	carnitine palmityl transferase (I).
26	Malonyl CoA formation is essential for the synthesis of _____	ketone bodies	fatty acid synthesis	cholestrol	lipoprotiens	fatty acid synthesis
27	Acyl CoA synthases are found _____	on the membrane of mitochondria	in the endoplasmic reticulum	peroxisomes	all the above	on the membrane of mitochondria
28	Oxidation of the fattyacids with odd no of carbon yields _____	acetyl CoA	acetyl CoA plus propionyl CoA	succinyl CoA	propionyl CoA	acetyl CoA plus propionyl CoA
29	Lipoprotein lipase is found in_____.	Spleen	renal medulla	adepose tissue	wall of blood capillaries	wall of blood capillaries
30	Phospholipase C attacks _____	the ester bond in the position 1	the ester bond in the position 2	hydrolyses both acyl groups	the ester bond in position 3	the ester bond in position 3
31	HMG-CoA reductase activity is increased by _____	thyroid hormone	glucagon	glucocortoid	Insulin	Insulin
32	Acetyl CoA carboxylase is converted into its inactive form by_____	palmitoyl CoA	cAMP dep.protein kinase	protein kinase .	phosphatase	cAMP dep.protein kinase
33	Cholesterol biosynthesis in stimulated by _____	glucogans	ephinephrine	nor-ephinephrine	Insulin	Insulin
34	During starvation the activity of _____ enzyme depresses	HMG CoA reductase	cAMP dep.protein kinase	protein kinase .	phosphatase	HMG CoA reductase
35	Long chain fatty acylCoA allosterically inhibits _____	pyrvate DH complex	citrate synthase	α-ketoglutarateDHcomplex	malateDH	citrate synthase

36	Lovastatin is a competitive inhibitor of _____	HMG-CoA reductase	HMG-CoA synthase	thiolase	all the above	HMG-CoA reductase
37	Phospholipase A2 is anenzyme which removes a fatty acid residue from lecithin to form_____	lecithin fragments	phosphatidic acids	glyceryl phosphate	lysolecithin	lysolecithin
38	Aspartate amino transferase use the following transamination _____	glutamic acid and pyruvic acid	glutamic acid and oxaloacetic acid	aspartic acid and pyruvic acid	aspartic acid and β-keto adipic acid	glutamic acid and oxaloacetic acid
39	The most abundant fatty acid in animal fat is _____	palmitic acid	acetic acid	DHA	EFA	palmitic acid
40	Rats feed with a fat free diet from birth could be deficient in_____	sphongolipid	TGL	phospholipid	prostagladins	TGL
41	Backbone of sphingolipids	sphingosine	lecithin	Glycerol	Plasmologen	sphingosine
42	The major fuel utilized by brain during fasting _____	glucose	fatty acid	protein	ketone bodies	ketone bodies
43	Bridge between the citric acid cycle and the urea cycle	fumarate	citrate	malate	argino sucinate	fumarate
44	Cofactors for acetylCoA carboxykase are following except	Biotin	Bicarbonate	NADH	Mn2+	NADH
45	Fatty acid synthase complexcontain	6 enzymes+2 acyl carrier proteins	6 enzymes+2 acyl carrier proteins	7 enzymes+1 acyl carrier proteins	7 enzymes+2 acyl carrier proteins	7 enzymes+1 acyl carrier proteins
46	Carboxylgroupdonor for conversion of acetyl CoA to malonylCoA is	Biotin	Bicarbonate	carbon dioxide	ATP	Bicarbonate
47	Beta oxidation means	Removal of 1C units	Removal of 2C units	Removal of 3C units	Removal of 4C units	Removal of 2C units
48	Cofactor for fattyacyl CoA synthetase is	Mn2+	Mg2+	Cu2+	Zn2+	Mg2+
49	Oxidation of Odd numbered fattyacidyields acetylCoA+_____	Butyrate	Propionate	succinate	Malonate	Propionate

50	Complete oxidation of one palmitic acid yields_____ATP	121	129	131	96	129
51	Acetyl CoA carboxylase enzyme is activated by _____	citrate	glucagons	ephinephrine	palmitylCoA	citrate
52	Elongation of fatty acid occurs in	Mitochomdria	Endoplasmic reticulam	Cytosol	golgi complex	Endoplasmic reticulam
53	Free fatty acid binding protein in plasma is	Globulin	Albumin	fibrinogen	fatty acid binding protein	Albumin
54	Most important oxidative pathway for fatty acid oxidation is	alpha oxidation	beta oxidation	omega oxidation	gamma oxidation	beta oxidation
55	Very long chain fatty acids are oxidized in	Mitochomdria	Endoplasmic reticulam	peroxisomes	golgi complex	peroxisomes
56	Rate limiting enzyme of Phosphatidyl choline synthesis is	Choline kinase	Phospho choline cytidyl transferase	Diaclglycerol Phospho choline transferase	Methyl transferase	Diaclglycerol Phospho choline transferase
57	Rate limiting enzyme of Phosphatidyl etanolamine synthesis is	Choline kinase	Phospho ethanloamine cytidyl transferase	Diaclglycerol Phospho ethanolamine transferase	Methyl transferase	Diaclglycerol Phospho ethanolamine transferase
58	Which phospholipid acts as second messenger	Phosphatidyl choline	Phosphatidyl serine	Phosphatidyl ethanloamine	Phosphatidyl Inositol	Phosphatidyl Inositol
59	Which enzyme cleaves the R1 ester bond of TAG	Phospholipase A1	Phospholipase A2	Phospholipase C	Protein kinase	Phospholipase A1
60	Which enzyme cleaves the R2 ester bond of TAG	Phospholipase A1	Phospholipase A2	Phospholipase C	Protein kinase	Phospholipase A2



UNIT-IV

SYLLABUS

Amino acid metabolism: Regulation of synthesis of pyruvate, serine, glutamate, aspartate, aromatic and histidine family of amino acids (Flow chart only) . Key role of glutamate dehydrogenase and glutamine synthetase in nitrogen metabolism and their allosteric regulations. Amino acid degradation- Oxidative deamination, Non oxidative deamination,decarboxylation and transamination. Ammonia formation and disposal- urea cycle and its regulation. Catabolism of carbon skeleton of amino acids.Biosynthesis of heme (porphyrin) and its regulations. Molecules derived from amino acids. Metabolic disorders- Alkaptonuria, phenyl ketonuria

REGULATION OF SYNTHESIS OF ASPARATE FAMILY OF AMINO ACIDS

Aspartate is non-essential in mammals, being produced from oxaloacetate by transamination. It can also be made in the Urea Cycle from Ornithine and Citrulline. In plants and microorganisms, aspartate is the precursor to several amino acids, including four that are essential for humans: methionine, threonine, isoleucine, and lysine.

Synthesis

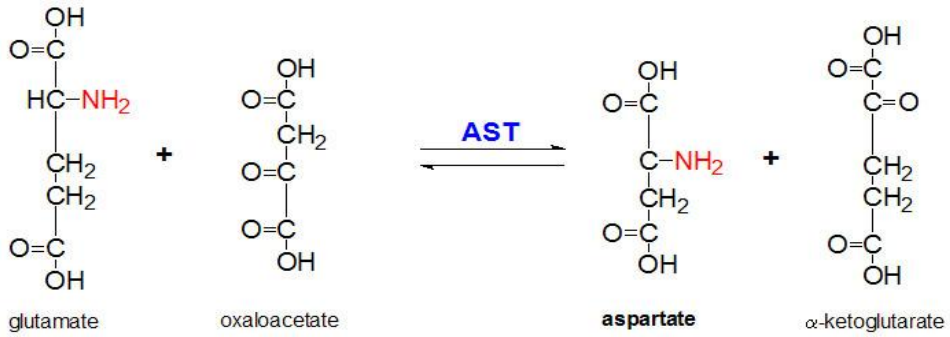
The conversion of aspartate to these other amino acids begins with reduction of aspartate to its "semialdehyde," $O_2CCH(NH_2)CH_2CHO$.[4] Asparagine is derived from aspartate via transamidation:

$-O_2CCH(NH_2)CH_2CO_2- + GC(O)NH_3+ O_2CCH(NH_2)CH_2CONH_3+ + GC(O)O$

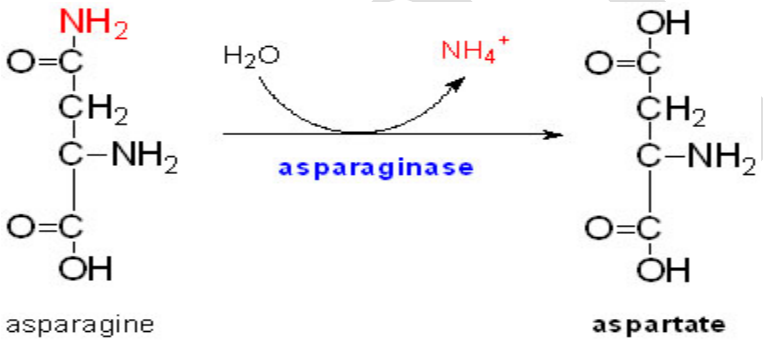
(where $GC(O)NH_2$ and $GC(O)OH$ are glutamine and glutamic acid, respectively)

Aspartate is also a metabolite in the urea cycle and participates in gluconeogenesis. It carries reducing equivalents in the malate-aspartate shuttle, which utilizes the ready interconversion of aspartate and oxaloacetate, which is the oxidized (dehydrogenated) derivative of malic acid. Aspartate donates one nitrogen atom in the biosynthesis of inosine, the precursor to the purine bases.

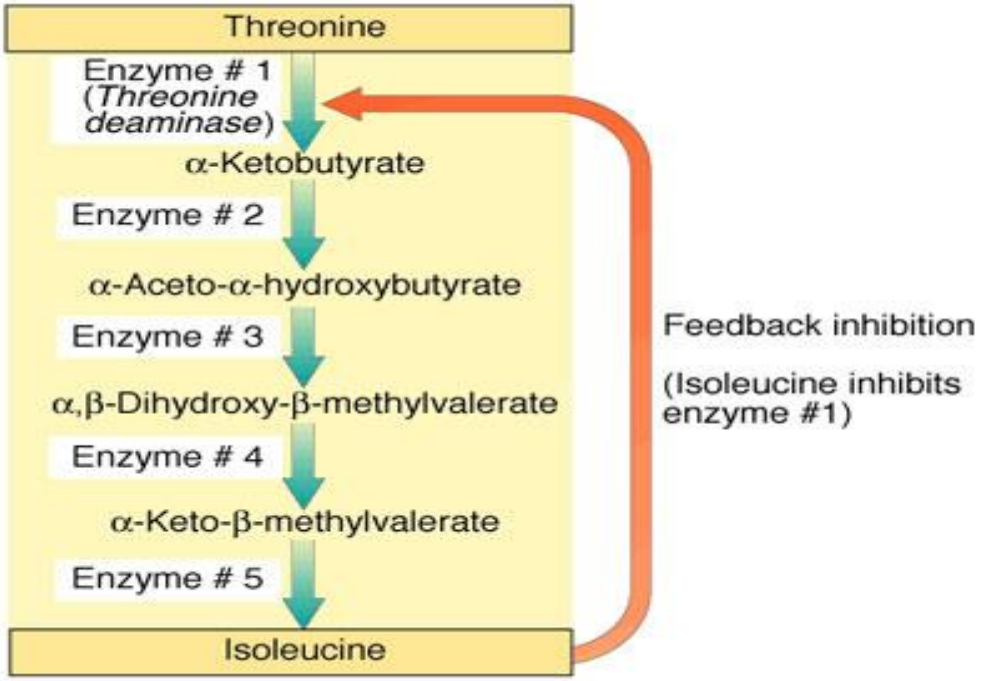
Aspartate is synthesized by a simple 1-step transamination reaction catalyzed by aspartate aminotransferase, AST (formerly referred to as serum glutamate-oxalate transaminase, SGOT)



Aspartate can also be derived from asparagine through the action of asparaginase



Synthesis and regulation of other aspartate family amino acids



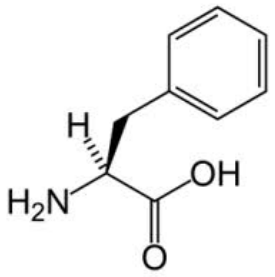
Synthesis and regulation of aromatic family of aminoacid

Aromatic amino acids are amino acids which include an aromatic ring.

Examples include:: phenylalanine, tryptophan, and tyrosine

Phenylalanine, histidine and tryptophan are essential amino acids since they are not synthesized in the human body, they must be derived from the diet. Tyrosine is semi-essential; it can be synthesized, but only from phenylalanine. A lack of the enzyme phenylalanine hydroxylase used in tyrosine synthesis causes phenylketonuria. Many plants and microorganisms synthesize aromatic amino acids. Many herbicides inhibit aromatic acid synthesis which is why they are ok around pets and humans

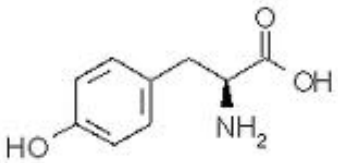
Phenylalanine (abbreviated as **Phe** or **F**) is an α-amino acid with the formula $C_6H_5CH_2CH(NH_2)COOH$. This essential amino acid is classified as nonpolar because of the hydrophobic nature of the benzyl side chain. Phenylalanine is a precursor for tyrosine, the monoamine signaling molecules dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), and the skin pigment melanin.. It is a direct precursor to the neuromodulator phenylethylamine, a commonly used dietary supplement.



Tryptophan (IUPAC-IUBMB abbreviation: **Trp** or **W**; IUPAC abbreviation: L-Trp or D-Trp; sold for medical use as **Tryptan**) is one of the 20 standard amino acids, as well as an essential amino acid in the human diet. It is an essential amino acid as demonstrated by its growth effects on rats.

For many organisms (including humans), tryptophan is an essential amino acid. This means that it cannot be synthesized by the organism and therefore must be part of its diet. Amino acids, including tryptophan, act as building blocks in protein biosynthesis.

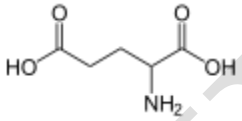
Tyrosine (abbreviated as **Tyr** or **Y**)[or **4-hydroxyphenylalanine**, is one of the 20 amino acids that are used by cells to synthesize proteins. A tyrosine residue also plays an important role in photosynthesis. In chloroplasts (photosystem II), it acts as an electron donor in the reduction of oxidized chlorophyll. In this process, it undergoes deprotonation of its phenolic OH-group. This radical is subsequently reduced in the photosystem II by the four core manganese clusters.



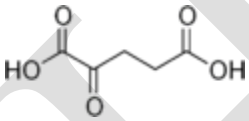
tyr y Tyrosin

Glutamate dehydrogenase

Glutamate dehydrogenase is an enzyme, present in most microbes and the mitochondria of eukaryotes, as are some of the other enzymes required for urea synthesis, that converts glutamate to α-Ketoglutarate, and vice versa. In animals, the produced ammonia is, however, usually bled off to the urea cycle. In bacteria, the ammonia is assimilated to amino acids via glutamate and amidotransferases. In plants, the enzyme can work in either direction depending on environment and stress Transgenic plants expressing microbial GDHs are improved in tolerance to herbicide, water deficit, and pathogen infections. They are more nutritionally valuable.



Glutamate



α-Ketoglutarate

The enzyme represents a key link between catabolic and metabolic pathways, and is, therefore, ubiquitous in eukaryotes.

Role in flow of nitrogen

Ammonia incorporation in animals and microbes occurs through the actions of glutamate dehydrogenase and glutamine synthetase. Glutamate plays the central role in mammalian and microbe nitrogen flow, serving as both a nitrogen donor and a nitrogen acceptor.

Regulation of glutamate dehydrogenase

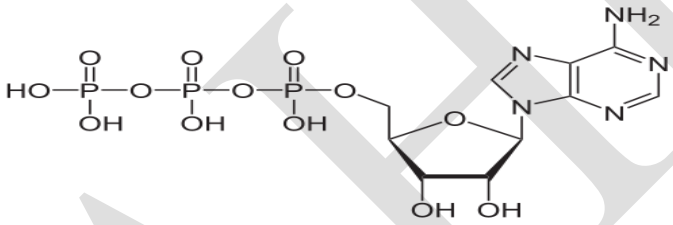
In Humans, the activity of glutamate dehydrogenase is controlled through ADP-ribosylation, a covalent modification carried out by the gene sirt4. This regulation is relaxed in response to caloric restriction and low blood glucose. Under these circumstances, glutamate dehydrogenase activity is raised in order to increase the amount of α-Ketoglutarate produced, which can be used to provide energy by being used in the citric acid cycle to ultimately produce ATP.

In microbes, the activity is controlled by the concentration of ammonium and or the like-sized Rubidium ion, which binds to an allosteric site on GDH and change the K_m (Michaelis constant) of the enzyme.[5]

The control of GDH through ADP-ribosylation is particularly important in insulin-producing β cells. Beta cells secrete insulin in response to an increase in the ATP:ADP ratio, and, as amino acids are broken down by GDH into α-ketoglutarate, this ratio rises and more insulin is secreted. SIRT4 is necessary to regulate the metabolism of amino acids as a method of controlling insulin secretion and regulating blood glucose levels.

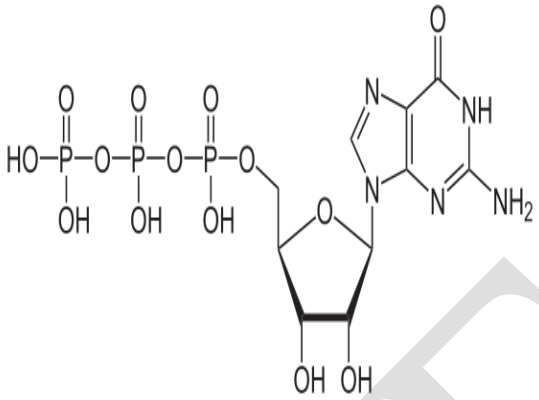
Regulation of biosynthesis

Adenosine triphosphate (ATP) production in an aerobic eukaryotic cell is tightly regulated by allosteric mechanisms, by feedback effects, and by the substrate concentration dependence of individual enzymes within the glycolysis and oxidative phosphorylation pathways. Key control points occur in enzymatic reactions that are so energetically favorable that they are effectively irreversible under physiological conditions.

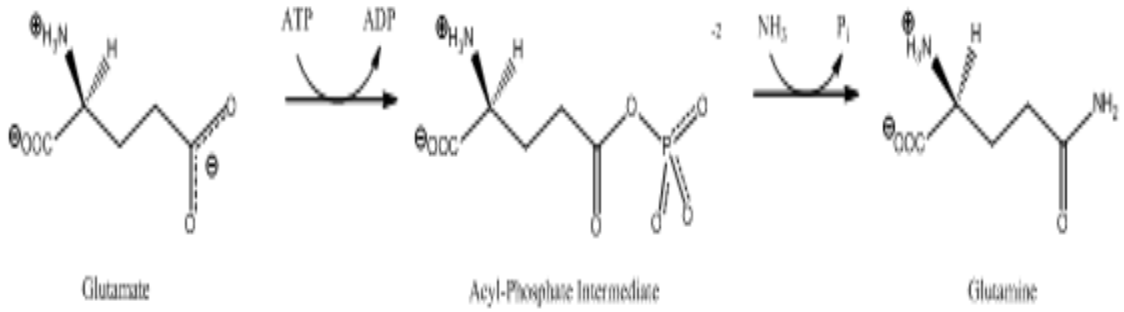


In glycolysis, hexokinase is directly inhibited by its product, glucose-6-phosphate, and pyruvate kinase is inhibited by ATP itself. The main control point for the glycolytic pathway is phosphofructokinase (PFK), which is allosterically inhibited by high concentrations of ATP and activated by high concentrations of AMP. The inhibition of PFK by ATP is unusual, since ATP is also a substrate in the reaction catalyzed by PFK; the biologically active form of the enzyme is a tetramer that exists in two possible conformations, only one of which binds the second substrate fructose-6-phosphate (F6P). A number of other small molecules can compensate for the ATP-induced shift in equilibrium conformation and reactivate PFK, including cyclic AMP, ammonium ions, inorganic phosphate, and fructose 1,6 and 2,6 biphosphate.

Guanosine-5'-triphosphate (**GTP**) is a purine nucleotide. It can act as a substrate for the synthesis of RNA during the transcription process. Its structure is similar to that of the guanine nucleobase, the only difference being that nucleotides like GTP have a ribose sugar and three phosphates, with the nucleobase attached to the 1' and the triphosphate moiety attached to the 5' carbons of the ribose.



Glutamine synthetase



Glutamine Synthetase uses ammonia produced by nitrate reduction, amino acid degradation, and photorespiration. The amide group of glutamate is a nitrogen source for the synthesis of glutamine pathway metabolites.

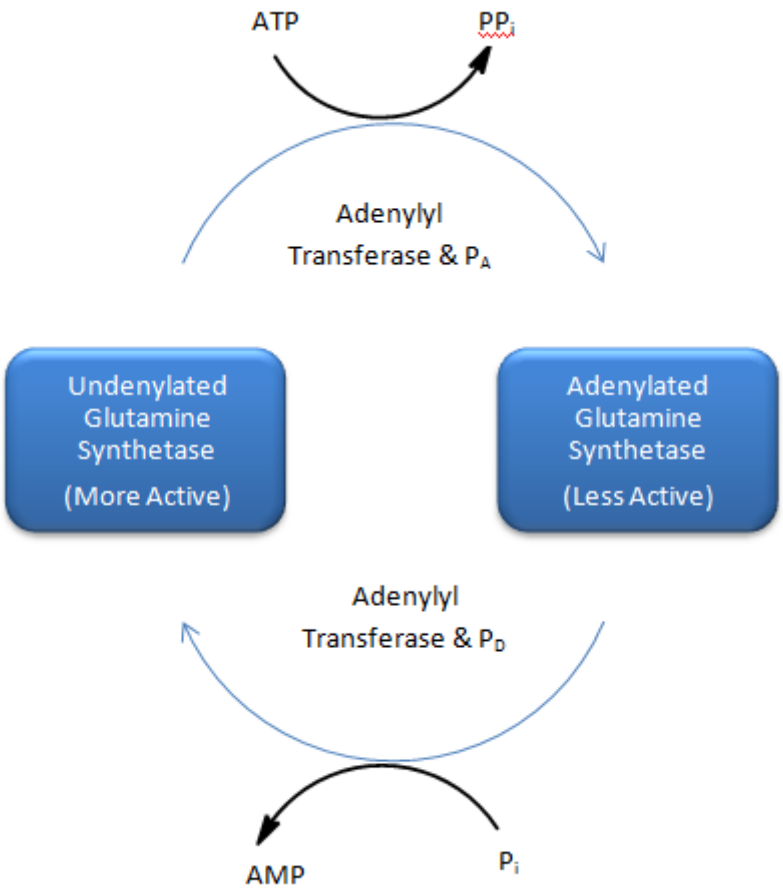
Biological Function

GS is present predominantly in the brain, kidneys, and liver. GS in the brain participates in the metabolic regulation of glutamate, the detoxification of brain ammonia, the assimilation of ammonia, recyclization of neurotransmitters, and termination of neurotransmitter signals. GS, in the brain, is found primarily is astrocytes. Astrocytes protect neurons against excitotoxicity by taking up excess ammonia and glutamate. In hyperammonemic environments (high levels of ammonia), astroglial swelling occurs. Different perspectives have approached the problem of astroglial swelling. One study shows that morphological changes occur that increase GS expression in glutamatergic areas or other adaptations that alleviates high levels of glutamate and ammonia. Another perspective is that astrocyte swelling is due to glutamine accumulation. To

prevent increased levels of cortical glutamate and cortical water content, a study has been conducted to prevent GS activity in rats by the use of MSO.

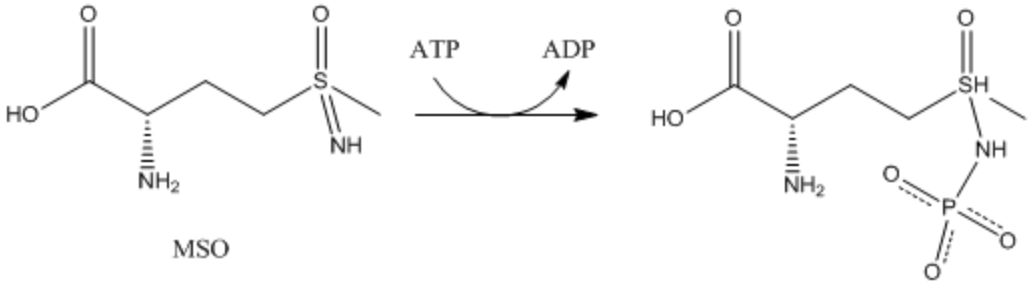
Regulation & Inhibition

Reversible Covalent Modification. A tyrosine residue in each subunit in GS can be modified by adenylylation. Adenylyl transferase catalyzes the adenylylation and phosphorolysis reactions. Adenyl transferase activity is influenced by two regulatory proteins: P_A and P_D. P_A reduces GS activity by attaching an AMP unit to GS. Adenylyl transferase and P_D removes the AMP unit. PA and PD may be interconverted via uridylyl transferase. Adenylylated GS is less active than unadenylated GS In the majority of gram-negative bacteria, GS can be modified by adenylylation (some cyanobacteria and green algae or exceptions).



Feedback regulation distinguishes the difference between two eukaryotic types of GS: brain and non-brain tissues. Non-brain GS responds to end-product feedback inhibition, while brain GS

does not. High concentrations of glutamine-dependent metabolites should inhibit GS activity, while low concentrations should activate GS activity.



MSO

Methionine Sulfoximine acting as an inhibitor to the glutamate binding site.

Inhibitors:

- Methionine Sulfoximine (MSO): MSO is an inhibitor that binds to the glutamate site. Bound to GS, MSO is phosphorylated by ATP that results in an irreversible, non-covalent inhibition of GS. The S-isomer configuration is more inhibitoryGlutamate entry is blocked into the active site by a stabilization of the flexible loop in the active site by MSO
- Phosphinothricin (PPT, Glufosinate): Phosphinothricin is an inhibitor that binds to the glutamate site. Glufosinate is used as an herbicide. Glufosinate treated plants die due to a buildup of ammonia and a cessation of photosynthesis
- Many synthetic inhibitors are available today.

Amino acid degradation

All amino acids contain at least one nitrogen atom, which forms their α-amino group. Some amino acids contain additional nitrogen atoms in their side chains. Nitrogen has no use in energy metabolism and has to be eliminated. There are two key processes in metabolic nitrogen elimination:

1. Transamination removes the α-amino group from one amino acid and transfers it to α-ketoglutarate. This leads to the accumulation of glutamate.
2. The release of nitrogen from glutamate and its conversion to urea. This is accomplished by the urea cycle in the liver.

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,

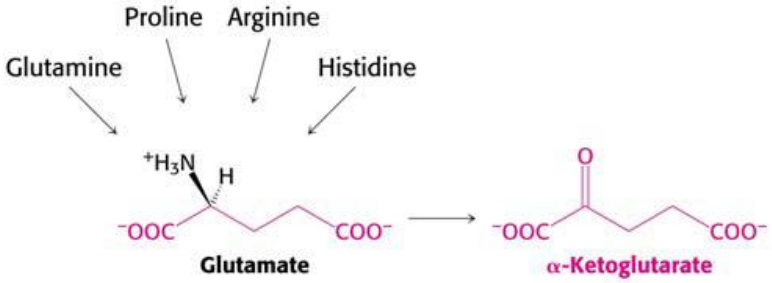
i. Pyruvate ii. Acetyl CoA iii. Acetoacetyl CoA iv. -α-Ketoglutarate v. Succinyl CoA vi. Fumarate vii. Oxaloacetate -ketoglutarate, succinyl CoA, fumarate and oxaloacetate can serve as α Pyruvate, precursors for glucose synthesis through gluconeogenesis. Amino acids giving rise to these intermediates 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.

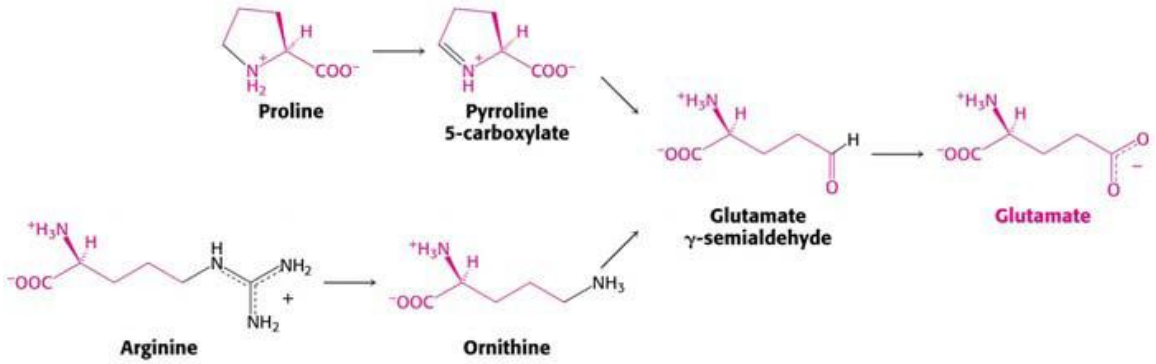
Glucogenic amino acids can be degraded to pyruvate or an intermediate in the Krebs Cycle. They are named glucogenic because they can produce glucose under conditions of low glucose. This process is also known as gluconeogenesis, or the production of "new glucose." Amino acids form glucose through degradation to pyruvate or an intermediate in the Krebs Cycle.

Amino Acids Degraded to α-Ketoglutarate.

Glutamine, proline, arginine and histidine are converted into glutamate which is then deaminated by a transaminase to form α-ketoglutarate.

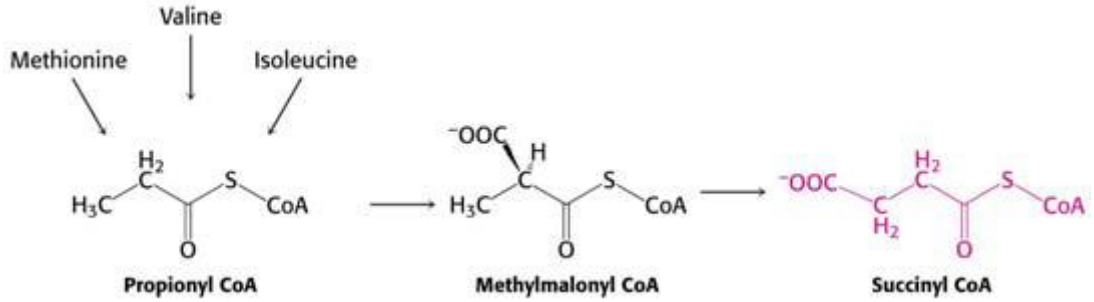


Glutamine is converted into glutamate by glutaminase. Proline is oxidized by proline oxidase to form pyrroline 5-carboxylate which spontaneously hydrolyzes to from glutamate γ-semialdehyde. From the urea cycle we know that arginase converts arginine into ornithine and urea. Ornithine δ-aminotransferase transfers the δ-amino group of ornithine to α-ketoglutarate to form glutamate γ-semialdehyde and glutamate.

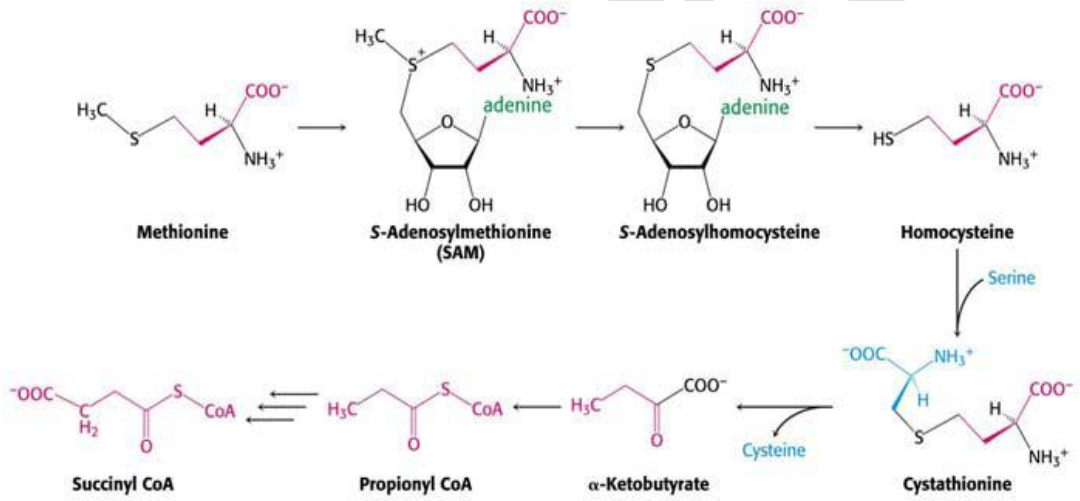


Amino Acids that are Broken Down in Succinyl-CoA.

Methionine, valanine and isoleucine are broken down into propoinyl CoA. By studying β-oxidation of odd chain fatty acids we know that propionyl CoA is converted into D-methylmalonyl CoA by propionyl CoA carboxylase. D-methylmalonyl CoA is racemized into L-methylmalonyl CoA by methylmalonyl CoA racemase. Methylmalonyl mutase produces succinyl CoA.



The first step is catalyzed by methionine adenosyl transferase which tranfers the adenosyl group of ATP to the sulfer of methionine to form SAM. Sam methylase transfers the activated methyl group to an acceptor to form S-adenosylhomocysteine which is hydrolyzed by adenosylhomocysteinase to form homocysteine. Cystathionine β-synthase is a PLP dependent enzyme that catalyzes the condensation of a serine residue with homocysteine to form cystathionine



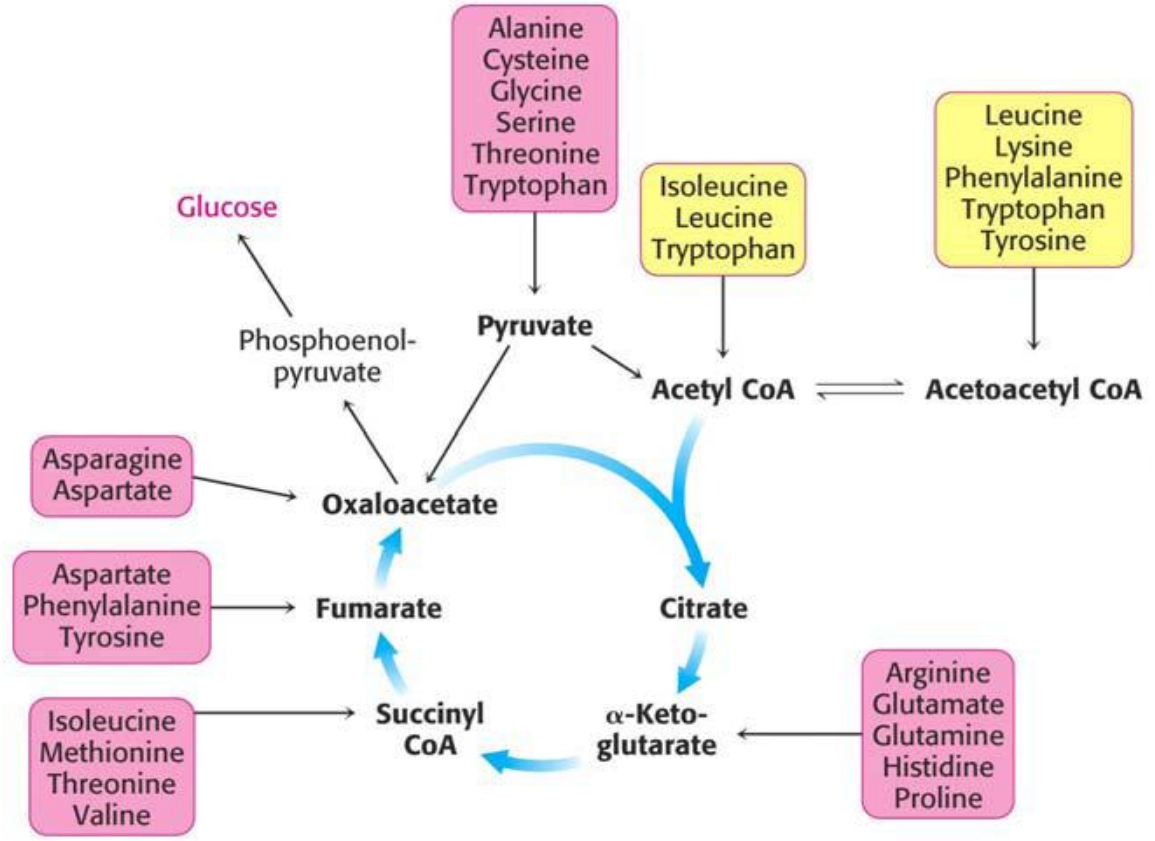


Fig: Amino acid degradation to intermediates of TCA cycle

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.

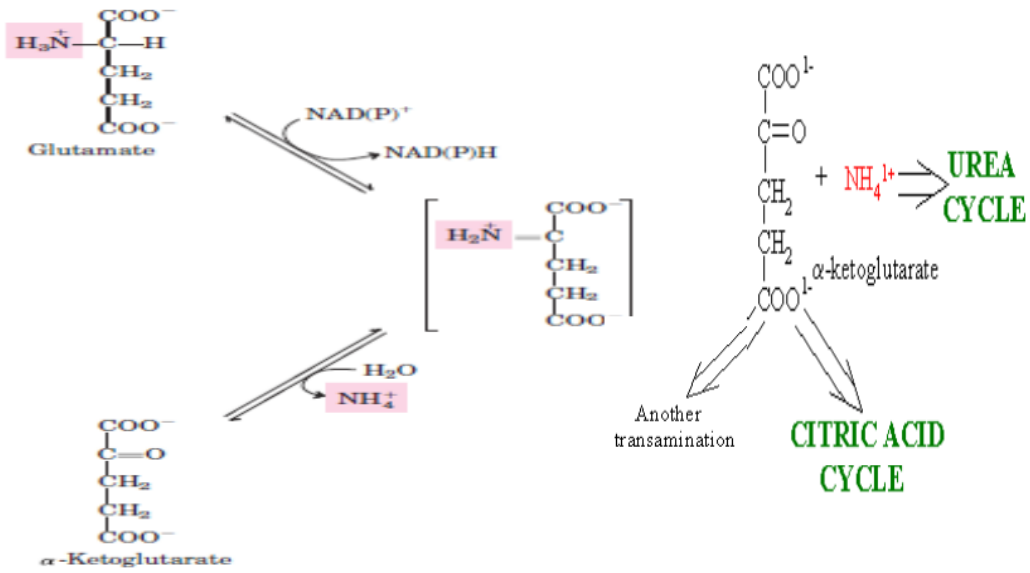
• It is one of few enzymes that can use either NAD or NADP as cofactor.

• 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.

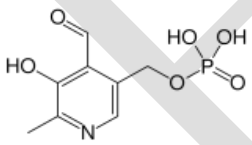
Non-oxidative Deamination

- 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.

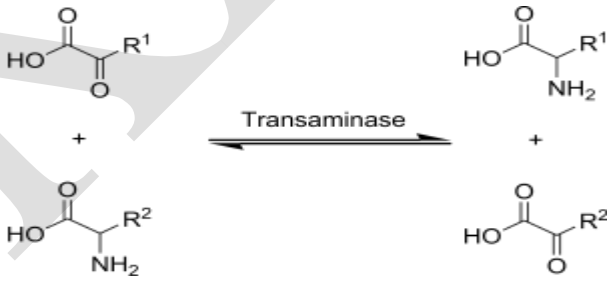
Transamination

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

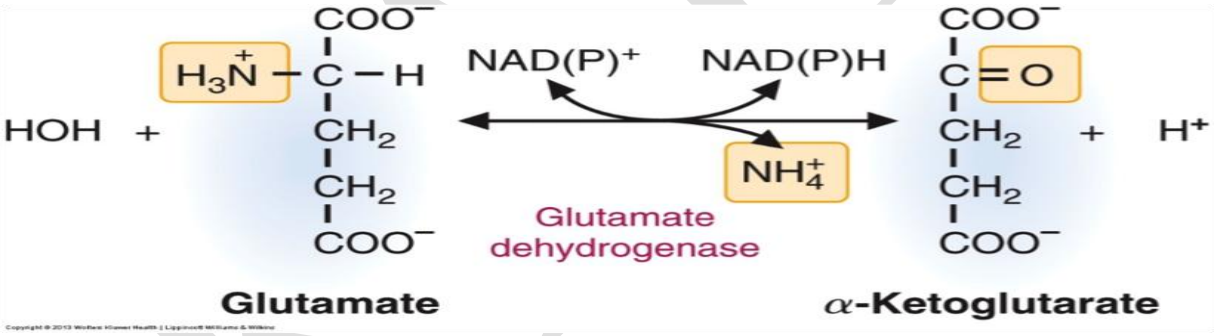
DECARBOXYLATION

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.

UREA CYCLE

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:

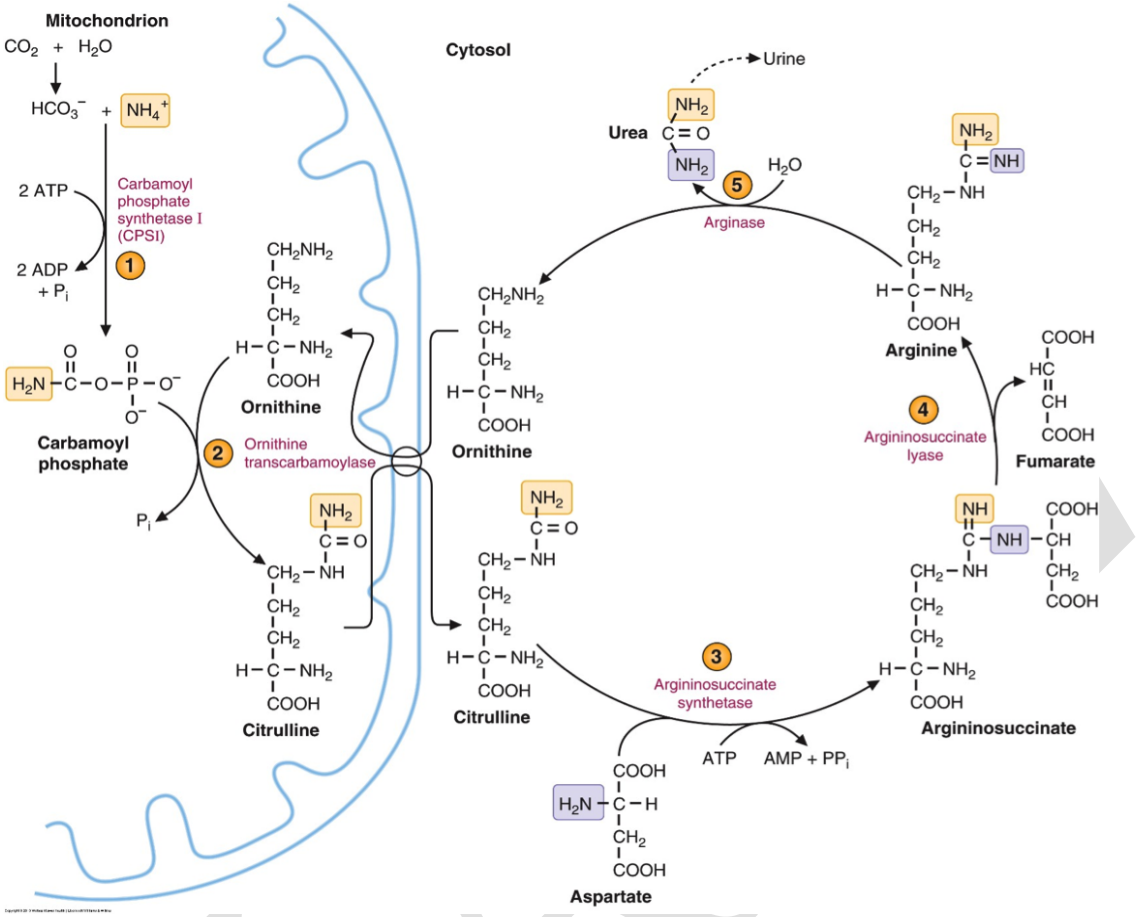
Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).

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

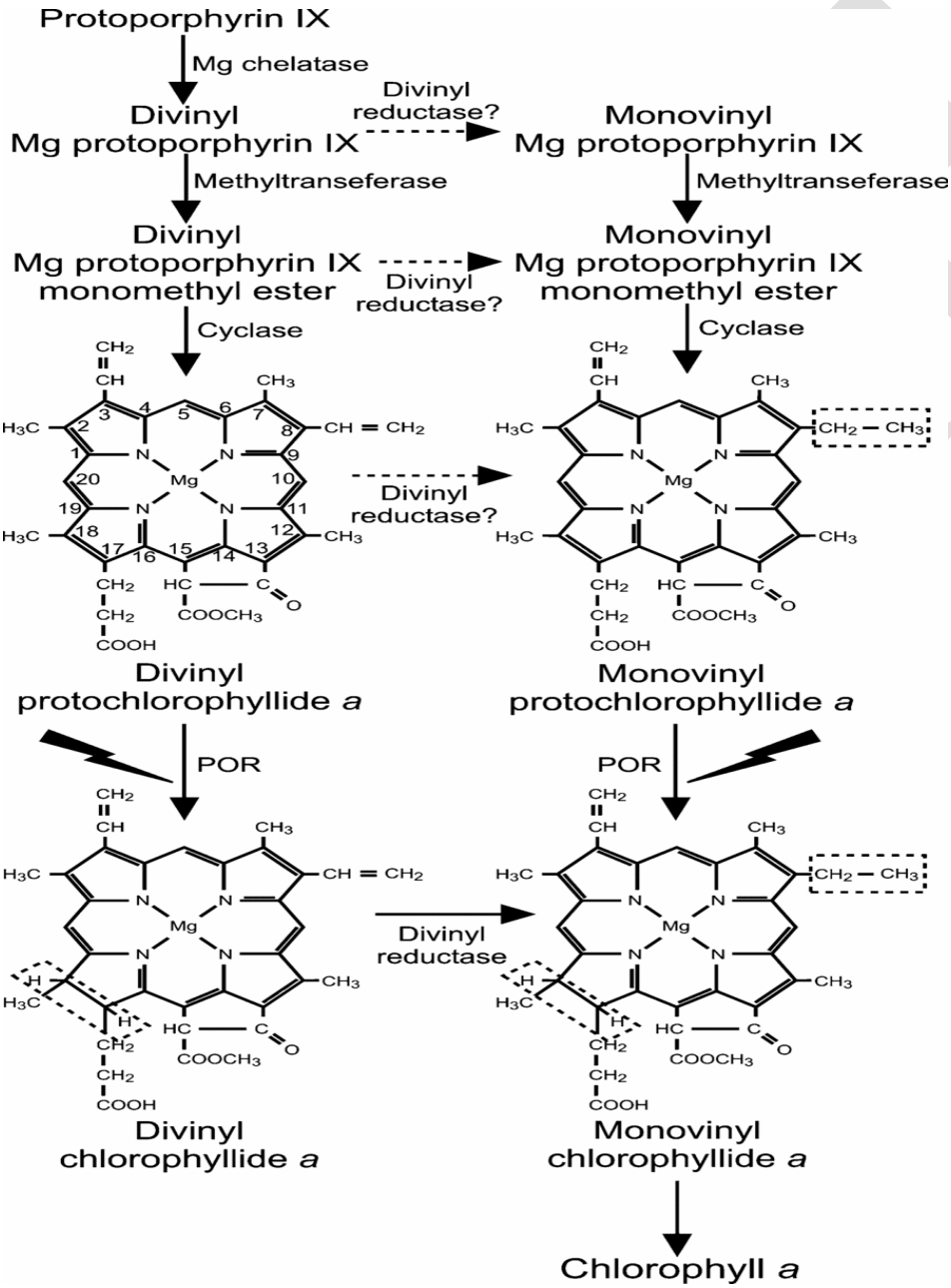
Regulation of urea cycle

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.

Biosynthesis of Chlorophyll and Haemoglobin

Chlorophyll Biosynthesis

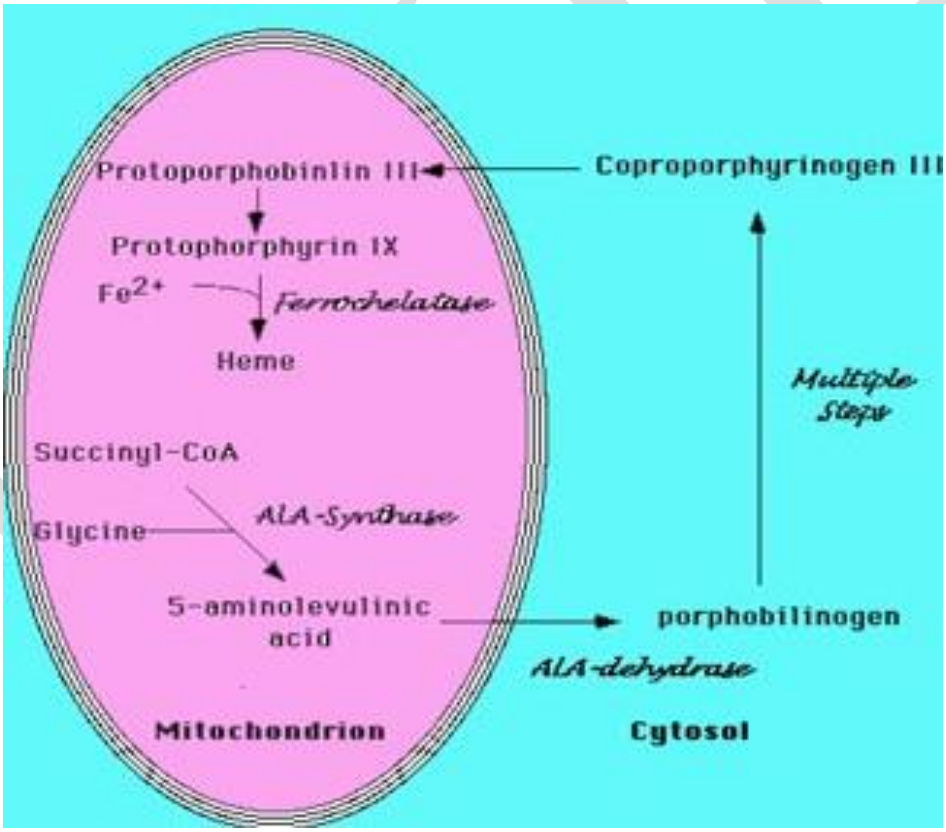


Hemoglobin Synthesis

Hemoglobin synthesis requires the coordinated production of heme and globin. Heme is the prosthetic group that mediates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the heme molecule.

Heme Synthesis

Heme is synthesized in a complex series of steps involving enzymes in the mitochondrion and in the cytosol of the cell (Figure 1). The first step in heme synthesis takes place in the mitochondrion, with the condensation of succinyl CoA and glycine by ALA synthase to form 5-aminolevulic acid (ALA). This molecule is transported to the cytosol where a series of reactions produce a ring structure called coproporphyrinogen III. This molecule returns to the mitochondrion where an addition reaction produces protoporhyrin IX.



The sythesis of heme is a complex process that involves multiple enzymatic steps. The process begins in the mitochondrion with the condensation of succinyl-CoA and glycine to form 5-

aminolevulinic acid. A series of steps in the cytoplasm produce coproporphrynogen III, which re-enters the mitochondrion. The final enzymatic steps produce heme.

The enzyme ferrochelatase inserts iron into the ring structure of protoporphyrin IX to produce heme. Deranged production of heme produces a variety of anemias. Iron deficiency, the world's most common cause of anemia, impairs heme synthesis thereby producing anemia. A number of drugs and toxins directly inhibit heme production by interfering with enzymes involved in heme biosynthesis. Lead commonly produces substantial anemia by inhibiting heme synthesis, particularly in children.

Globin Synthesis

Two distinct globin chains (each with its individual heme molecule) combine to form hemoglobin. One of the chains is designated alpha. The second chain is called "non-alpha". With the exception of the very first weeks of embryogenesis, one of the globin chains is always alpha. A number of variables influence the nature of the non-alpha chain in the hemoglobin molecule. The fetus has a distinct non-alpha chain called gamma. After birth, a different non-alpha globin chain, called beta, pairs with the alpha chain. The combination of two alpha chains and two non-alpha chains produces a complete hemoglobin molecule (a total of four chains per molecule).

The combination of two alpha chains and two gamma chains form "fetal" hemoglobin, termed "hemoglobin F". With the exception of the first 10 to 12 weeks after conception, fetal hemoglobin is the primary hemoglobin in the developing fetus. The combination of two alpha chains and two beta chains form "adult" hemoglobin, also called "hemoglobin A". Although hemoglobin A is called "adult", it becomes the predominate hemoglobin within about 18 to 24 weeks of birth.

The pairing of one alpha chain and one non-alpha chain produces a hemoglobin dimer (two chains). The hemoglobin dimer does not efficiently deliver oxygen, however. Two dimers combine to form a hemoglobin tetramer, which is the functional form of hemoglobin. Complex biophysical characteristics of the hemoglobin tetramer permit the exquisite control of oxygen uptake in the lungs and release in the tissues that is necessary to sustain life.

The genes that encode the alpha globin chains are on chromosome 16. Those that encode the non-alpha globin chains are on chromosome 11. Multiple individual genes are expressed at each site. Pseudogenes are also present at each location. The alpha complex is

called the "alpha globin locus", while the non-alpha complex is called the "beta globin locus". The expression of the alpha and non-alpha genes is closely balanced by an unknown mechanism. Balanced gene expression is required for normal red cell function. Disruption of the balance produces a disorder called thalassemia.

Alkaptouria

Alkaptonuria (**black urine disease** or **alcaptonuria**) is a rare inherited genetic disorder of phenylalanine and tyrosine metabolism. This is an autosomal recessive condition that is due to a defect in the enzyme homogentisate 1,2-dioxygenase (EC 1.13.11.5), which participates in the degradation of tyrosine. As a result, a toxic tyrosine byproduct called homogentisic acid (or alkapton) accumulates in the blood and is excreted in urine in large amounts (hence *-uria*). Excessive homogentisic acid causes damage to cartilage (ochronosis, leading to osteoarthritis) and heart valves as well as precipitating as kidney stones. Treatment with nitisinone, which suppresses homogentisic acid production, is being studied.[1] Alkaptonuria is more common in Slovakia and the Dominican Republic than in other countries.[2][3]

Signs and symptoms

Alkaptonuria is often asymptomatic, but the sclera of the eyes may be pigmented (often only at a later age),[1] and the skin may be darkened in sun-exposed areas and around sweat glands; sweat may be coloured brown. Urine may turn brown if collected and left exposed to open air, especially when left standing for a period of time. Kidney stones and stone formation in the prostate (in men) are common and may occur in more than a quarter of cases.[1]

The main symptoms of alkaptonuria are due to the accumulation of homogentisic acid in tissues. In the joints this leads to cartilage damage, specifically in the spine, leading to low back pain at a young age in most cases. Cartilage damage may also occur in the hip and shoulder. Joint replacement surgery (hip and shoulder) is often necessary at a relatively young age.[1]

Valvular heart disease, mainly calcification and regurgitation of the aortic and mitral valves, may occur, and in severe and progressive cases valve replacement may be necessary. Coronary artery disease may be accelerated in alkaptonuria.[1]

A distinctive characteristic of alkaptonuria is that ear wax exposed to air turns red or black (depending on diet) after several hours because of the accumulation of homogentisic acid.[4]

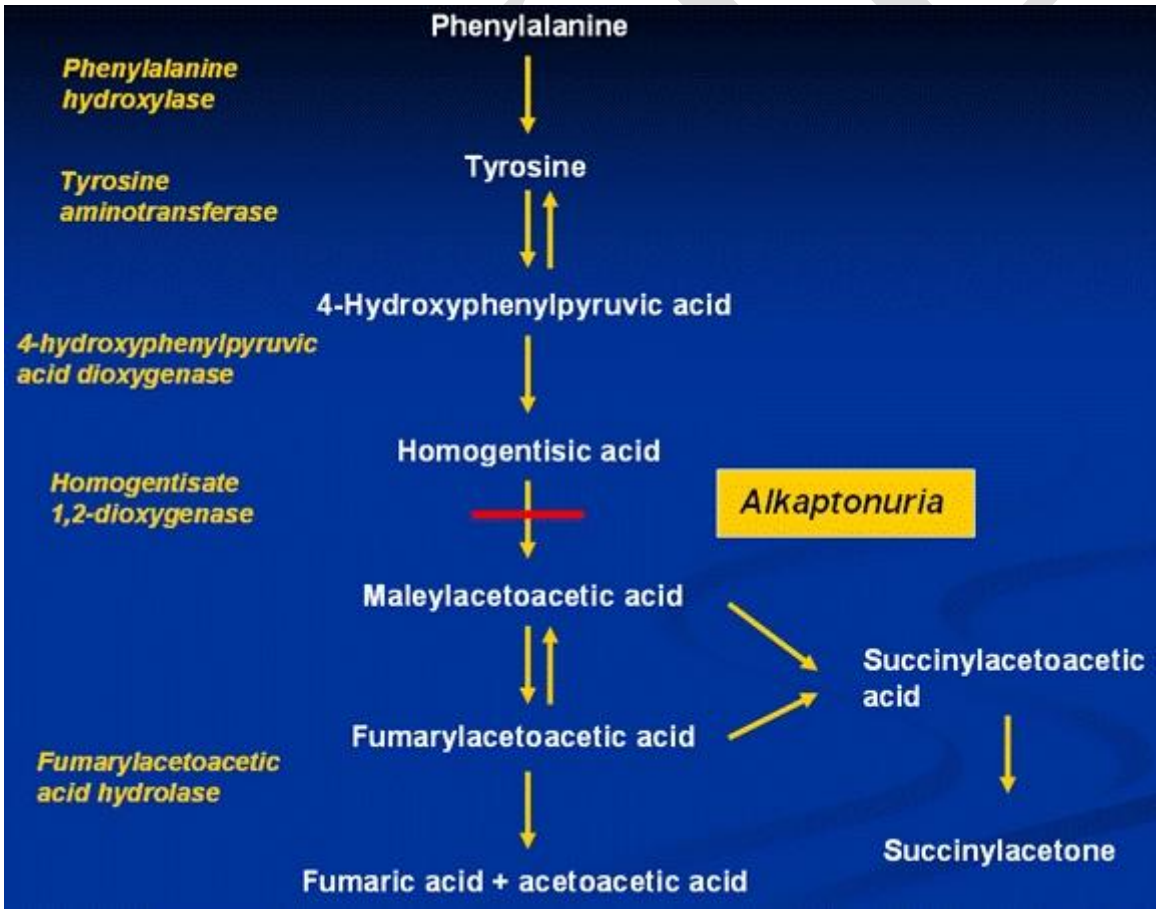
Diagnosis

. Both blood plasma and urine can be used for diagnosis. In healthy subjects, homogentisic acid is absent in both blood plasma and urine. In alkaptonuria, plasma levels are 6.6 micrograms/ml on average, and urine levels are on average 3.12 mmol/mmol of creatinine.[1]

Pathophysiology

Homogentisic acid is a natural intermediary of the metabolism of tyrosine, an amino acid. Hepatic homogentisate 1,2-dioxygenase (coded by the *HGD* gene) metabolises homogentisic acid into 4-maleylacetoacetate. Alkaptonuria arises in people who have inherited two abnormal *HGD* genes: one from each parent. Numerous different *HGD* mutations have been identified.[1]

In a patient who underwent a liver transplant for an unrelated problem, alkaptonuria resolved and joint disease stabilised after the transplant, confirming that the liver is the main site of homogentisic acid production in alkaptonuria.[5]



Phenylketonuria

Phenylketonuria (**PKU**) is an autosomal recessive metabolic genetic disorder characterized by an error in the genetic code for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional.[1]:541 This enzyme is necessary to metabolize the amino acid phenylalanine (Phe) to the amino acid tyrosine. When PAH enzymatic activity is reduced, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which is detected in the urine.[2]

Screening and presentation

Blood is taken from a two-week old infant to test for phenylketonuria

PKU is normally detected using the HPLC test, but some clinics still use the Guthrie test, part of national biochemical screening programs. Most babies in developed countries are screened for PKU soon after birth.[5] For HPLC, a cutoff of 30.0 mg/L may be used, with higher values defining phenylketonuria.[6]

If a child is not screened during the routine newborn screening test (typically performed 6 –14 days after birth, using samples drawn by neonatal heel prick), the disease may present clinically with seizures, albinism (excessively fair hair and skin), and a "musty odor" to the baby's sweat and urine (due to phenylacetate, one of the ketones produced). In most cases, a repeat test should be done at approximately two weeks of age to verify the initial test and uncover any phenylketonuria that was initially missed.

Untreated children are normal at birth, but fail to attain early developmental milestones, develop microcephaly, and demonstrate progressive impairment of cerebral function. Hyperactivity, EEG abnormalities and seizures, and severe learning disabilities are major clinical problems later in life. A "musty or mousy" odor of skin, hair, sweat and urine (due to phenylacetate accumulation); and a tendency to hypopigmentation and eczema are also observed.

In contrast, affected children who are detected and treated are less likely to develop neurological problems or have seizures and mental retardation, though such clinical disorders are still possible.

Tetrahydrobiopterin-deficient hyperphenylalaninemia

A rarer form of hyperphenylalaninemia occurs when PAH is normal, but there is a defect in the biosynthesis or recycling of the cofactor tetrahydrobiopterin (BH_4) by the patient.[10] This cofactor is necessary for proper activity of the enzyme. The coenzyme (called biopterin) can be supplemented as treatment.

Levels of dopamine can be used to distinguish between these two types. Tetrahydrobiopterin is required to convert phenylalanine to tyrosine, but it is also required to convert tyrosine to L-DOPA (via the enzyme tyrosine hydroxylase), which in turn is converted to dopamine. Low levels of dopamine lead to high levels of prolactin. By contrast, in classical PKU, prolactin levels would be relatively normal. Tetrahydrobiopterin deficiency can be caused by defects in four different genes. These types are known as HPABH4A, HPABH4B, HPABH4C, and HPABH4D.[11]

Metabolic pathways

The enzyme phenylalanine hydroxylase normally converts the amino acid phenylalanine into the amino acid tyrosine. If this reaction does not take place, phenylalanine accumulates and tyrosine is deficient. Excessive phenylalanine can be metabolized into phenylketones through the minor route, a transaminase pathway with glutamate. Metabolites include phenylacetate, phenylpyruvate and phenethylamine.[12] Elevated levels of phenylalanine in the blood and detection of phenylketones in the urine is diagnostic.

Phenylalanine is a large, neutral amino acid (LNAA). LNAAs compete for transport across the blood-brain barrier (BBB) via the large neutral amino acid transporter (LNAAT). If phenylalanine is in excess in the blood, it will saturate the transporter. Excessive levels of phenylalanine tend to decrease the levels of other LNAAs in the brain. However, as these amino acids are necessary for protein and neurotransmitter synthesis, Phe buildup hinders the development of the brain, causing mental retardation

POSSIBLE QUESTIONS

2 mark questions

1. Explain transamination with an example
2. Draw the flow chart of urea cycle
3. What is phenyl ketonuria? Add the symptoms of this disease.
4. Draw the reactions of urea cycle
5. How is hemoglobin synthesis regulated?
6. Explain the clinical condition alkaptonuria
7. Draw the regulation of aromatic family amino acid synthesis
8. How is chlorophyll synthesis regulated?
9. Draw the regulation of glutamine synthetase
10. Add note regulation of aliphatic family amino acid synthesis
11. List the aminoacids of asparatate family

Essay type questions (6Marks)

1. Explain the synthesis and regulation of hemoglobin
2. Explain the synthesis and regulation of Aspartate family of amino acids.
3. Explain the synthesis and regulation of Aromatic family of amino acids.
4. How are the synthesis of aspartate and aromatic family of amino acids controlled?
5. Discuss the synthesis and regulation of urea cycle
6. Explain the synthesis and regulation of chorophyll
7. Describe the Oxidative deamination and transamination reactions with an example

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I MSc BIOCHEMISTRY-Second Semester

REGULATION OF METABOLIC PATHWAYS (18BCP201)

UNIT-IV **MULTIPLE CHOICE QUESTIONS**

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Serine gives rise to _____	Tyrosine	Arginine and proline	arginine and glutamine	glycine and cysteine	glycine and cysteine
2	Aminoacid which yields single carbon pool except _____	serine	histidine	glycine	glutamic acid	glutamic acid
3	Glycine is involved in the synthesis of compounds except _____	glutamic acid	serine	haemoglobin	purine	glutamic acid
4	δ- ALA synthetase activity is increased in the individuals trated with drugs , barbiturate phenol, because it _____	catalysis a rate limiting reaction in the porphyrin synthesis	occurs in cytosol	inhibited by heavy metals ions such as lead	requires biotin as cofactor	catalysis a rate limiting reaction in the porphyrin synthesis
5	Formation of a imino acid intermediate is the characteristic of __	oxidative deamination	non oxidative deamination	transamination	transmethylation	oxidative deamination
6	Amino transferase enzyme for its activity required _____	pantothenate	niacin	pyridoxal phosphate	thiamine	pyridoxal phosphate
7	Arginine is converted to ornithine by arginase with the removal of _____	uric acid	uroconic acid	urea	uronic acid	urea
8	The end product of purine catabolism in other mammals except man_____	uric acid	ammonia	allantoin	creatinine	allantoin
9	Transamination is a _____--	reversible process	irreversible process	both of the above	none of the above	reversible process
10	Synthesis of glutamine accompanied by hydrolysis of _____	ATP	ADP	TPP	crteatine phosphate	ATP
11	The chief end product of purine catabolism in an _____	urea	uracil	uric acid	allantoin	uric acid
12	The key regulatory enzyme of heme biosynthesis _____	ALA synthase	porphyrin carboxylase	porphyrin synthase	lactate dehydrogenase	ALA synthase

13	Cytochrome synthase plays a role in the _____	TCA cycle	electron transport chain	HMP shunt	glycolysis	electron transport chain
14	Phenylalanine is converted into tyrosine by _____	glutamate dehydrogenasae	homogentisate oxidase	phenyl alanine hydrooxylase	tyrosine hydroxylase	phenyl alanine hydrooxylase
15	Which one of the following is the major end product of the nitrogen catabolism inhumans?	allantoin	urea	uric acid	ammonia	urea
16	Conversion of L- Glutamate to L-Glutamine is catalyzed by _____-	glutamine transferase	glutamine dehydrogenase	glutamine decarboxylase	glutamine synthetase	glutamine synthetase
17	Conversion of L-cystine to L-Cysteine is catalyzed by _____	cystein reductase	cystine synthetase	cystine dioxygenase	cysteine hydrolase	cystein reductase
18	Which one of the following enzyme is involved in the conversion of L-Methionine to SAdenosyl L-Methionine?	adenosyl transferase	methionine transferase	L-methionine Adenosyl transferase	none of the above	L-methionine Adenosyl transferase
19	ALA synthase requires_____as the cofactor	Biotin	Mg2+	pyridoxalphosphate	NAD	pyridoxalphosphate
20	Heme is synthesized from _____	Succlinyl CoA and valine	Succinyl CoA and glycine	SuccinylCoA and isoleucine	AcetylCoA and glycine	Succinyl CoA and glycine
21	ALA synthase present in_____	mitochondria	cytosol	both	endoplasmic reticulum	mitochondria
22	Liver glutamate dehydrogenase activity is activated by _____	ATP	GTP	ADP	NADH	ADP
23	α-ketoglutarate is an intermediate during the catabolism of _____	arginine	tyrosine	methionine	serine	arginine
24	The reaction catalyzed by carbomyl phosphate synthase I of urea cycle occurs in the -	cytosol of liver cells	matrix of mitochondria of liver cells.	cytosol of kidney	matrix of mitochondria of kidney cells	matrix of mitochondria of liver cells.
25	Synthesis of δ-amino levulinate _____is catalyzed by	ALA synthase	occurs in the liver mitochondria	requires succinyl CoA and glycine	inhibited by pyridoxal phosphate	inhibited by pyridoxal phosphate
26	Glycine is formed from _____	pyruvate	α-KG	aspartate	choline	pyruvate

27	The protein which is involved in the storage of O2 in the muscle_____	haemoglobin	catalase	cytochrome-C	myoglobin	myoglobin
28	The conversion of bilirubin into bilirubidindiglucuronide is mainly takes place in	kidney	blood	bileduct	hepatocytes	hepatocytes
29	_____ enzyme is defect in β-aminoisobutyric aciduria	transaminase	orotidyl decarboxylase	ornitine transcarbamylase	adenosine trans aminase	transaminase
30	The end product of phenylalanine is _____	fumarate and acetoacetate	fumarate and pyruvate	fumarate and oxaloacetate	succinylCoA and acetylCoA	fumarate and acetoacetate
3`	Prephenate is a common intermediate in the synthesis of _____	tryptophan and phenylalanine	phenylalanine and tyrosine	tryptophan and tyrosine	all the above	tryptophan and phenylalanine
32	The follwing compounds received an amide nitrogen from glutamine except _____	tryptophan	carbamylphosphate	alanine	AMP	alanine
33	Which one the following statement regarding arginine is false?	it is semi essential	it is glucogenic	it is the precursor of nitric oxide	it contains imidazole grou	it contains imidazole grou
34	The rate limiting reaction in the synthesis of heme in the liver is	ALA-synthase-	ALA-dehydratase	uroporphyrinogen-synthase	uroporphyrinogen-decarboxilase	ALA-synthase-
35	The substance formed by the combination of ammonia and carbondioxide is _____.	tryptophan	carbamoylphosphate	alanine	AMP	carbamoylphosphate
36	____ provide the carbon atom at position 4and 5 and nitrogen atom at 7 in purine.	tryptophan	glutamine	alanine	AMP	glutamine
37	An amino acid not involved in urea cycle _____	arginine	histidine	ornithine	citrulline	arginine
38	The coenzyme involved in transamination reaction _____	biotin	pyridoxal phosphate	NAD+	FMN	pyridoxal phosphate
39	An anaplerotic reaction which sustains availability of oxaloacetate is the carboxylation of	glutamate	pyruvate	citrate	succinate	pyruvate

40	Urea’s nitrogen atom are obtained from _____	ornithine and citrulline	ammonium and aspartate ions	amonium ions and argentine	aspartate and citrulline	ammonium and aspartate ions
41	Heme controls the activity of ALA synthase by _____	feed back inhibition	repression	inhibition of transport of ALA	all the above	repression
42	Lysine feed backly inhibits _____	asparto kinaseI	asparto kinaseII	aspartokinase III	all the above	asparto kinaseI
43	Channeling is observed in _____	tryptophan synthase	chorismate mutase	prophenate dehydrogenase	all the above	tryptophan synthase
44	Essential aminoaicds are	Valine	Leucine	Isoleucine	Trptophan	Trptophan
45	Phenyl alanine hydroxylase is present in	liver	kidney	Brain	Intestine	liver
46	Growing infants and pregnant womens are in	Positive nitrogen balance	Negative nitrogen balance	nitrogen equilibrium	none	Positive nitrogen balance
47	Turn over of protein in body is	1-2%	0.5-1%	2-3%	3-4%	1-2%
48	% of protein reutilized for synthetic purpose	60-40	80-100	75-80	95-100	75-80
49	% of protein degraded to urea in body during protein turnover is	30-40	20-25	60-40	75-80	20-25
50	Protein that target for many intracellular protein degradation	Ubiquitin	Cyt p450 MAO	lyase	Hydroxu\ylase	Ubiquitin
51	Excess aminoacids are always	stored	degraded	unaltered	none	degraded
52	_____ocupies central role in nitrogen metabolism	Glutamate synthetase	Glutamine synthetase	Glutamate dehrogenase	all	Glutamate dehrogenase
53	Urea is formed from all	Ammonia,CO2,Asp	Ammonia,CO2,Gln	Ammonia,HCO3,Asp	Gln,CO2,Asp	Ammonia,CO2,Asp
54	Rate limiting step in urea synthtesis	Ornithine trans carboxylase	Argino succinate synthetase	carbamoyl phosphate synthetase	arginase	carbamoyl phosphate synthetase
55	Precursor for heme	Succinyl CoA+Glycine	Succinyl CoA+PEP	PEP+Glycine	PEP+Erythrose PO4	Succinyl CoA+Glycine
56	Respiratory proteins that occur in muscle cell of vertebrate	Hemoglobin	Myoglobin	Immunoglobin	Gamma globulin	Myoglobin
57	Magnesium containing porphyrin is	Hemoglobin	Myoglobin	Chlorophyll	Cytochrome	Chlorophyll
58	Supressor of ALA synthase in heme biosynthesis	Glucose	iron	Glycine	Succinyl CoA	Glucose

59	Aliphatic family of Aminoacids are controlled through	Cumulative FBI	Concerted FBI	Sequntial FBI	All	Sequntial FBI
60	Most of the aminoacids are degraded into intermediates of _____	Glycolysis	TCA cycle	HMP shunt	Glyoxylate cycle	TCA cycle

UNIT-V

SYLLABUS

Nucleic acid metabolism: De novo synthesis of purine and its regulation – Role of PRPP amino transferase. De novo synthesis of pyrimidine and its regulation – Role of aspartate carbomyl transferase. Regulation of deoxy ribonucleotides by activators and inhibitors. Intergration of metabolism. Metabolism during starvation. Tissue specific metabolism- Metabolic profile of major organs- Brain, Muscle, Liver and Adipose tissue. Metabolic disorders- Gout, SCID.

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

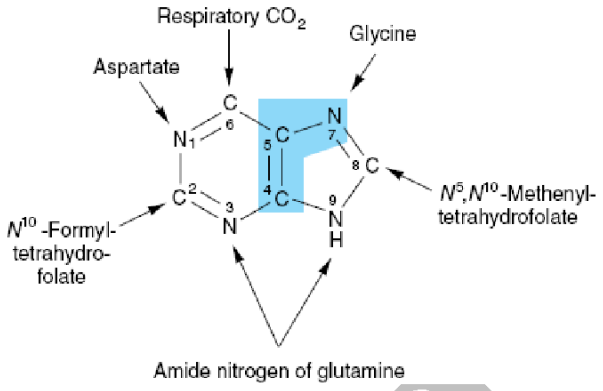
Purine and pyrimidine nucleotides are synthesized in vivo at rates consistent with physiologic need. Intracellular mechanisms sense and regulate the pool sizes of nucleotide triphosphates (NTPs), which rise during growth or tissue regeneration when cells are rapidly dividing. Early investigations of 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.

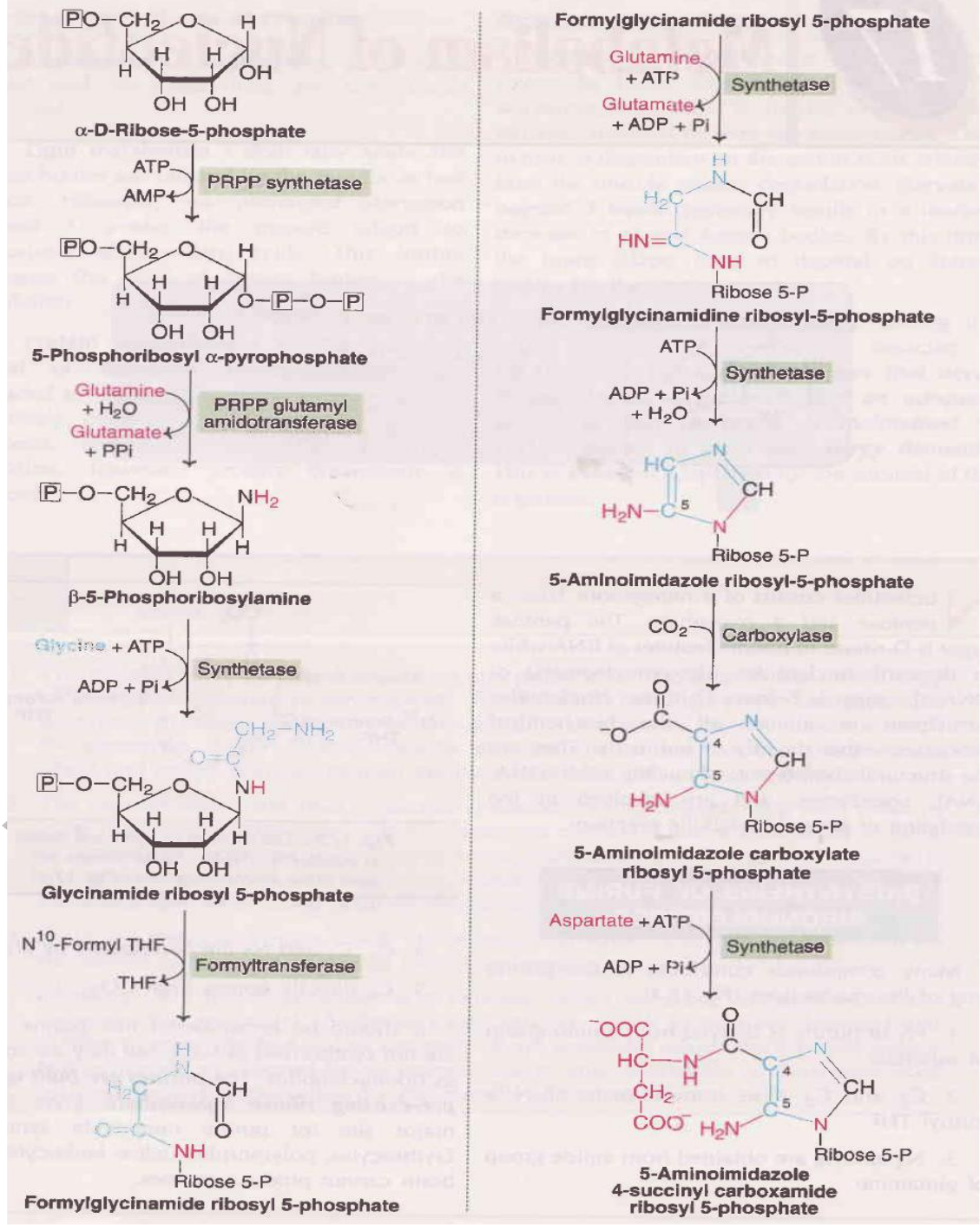
AMP & GMP Feedback-Regulate PRPP Glutamyl Amidotransferase

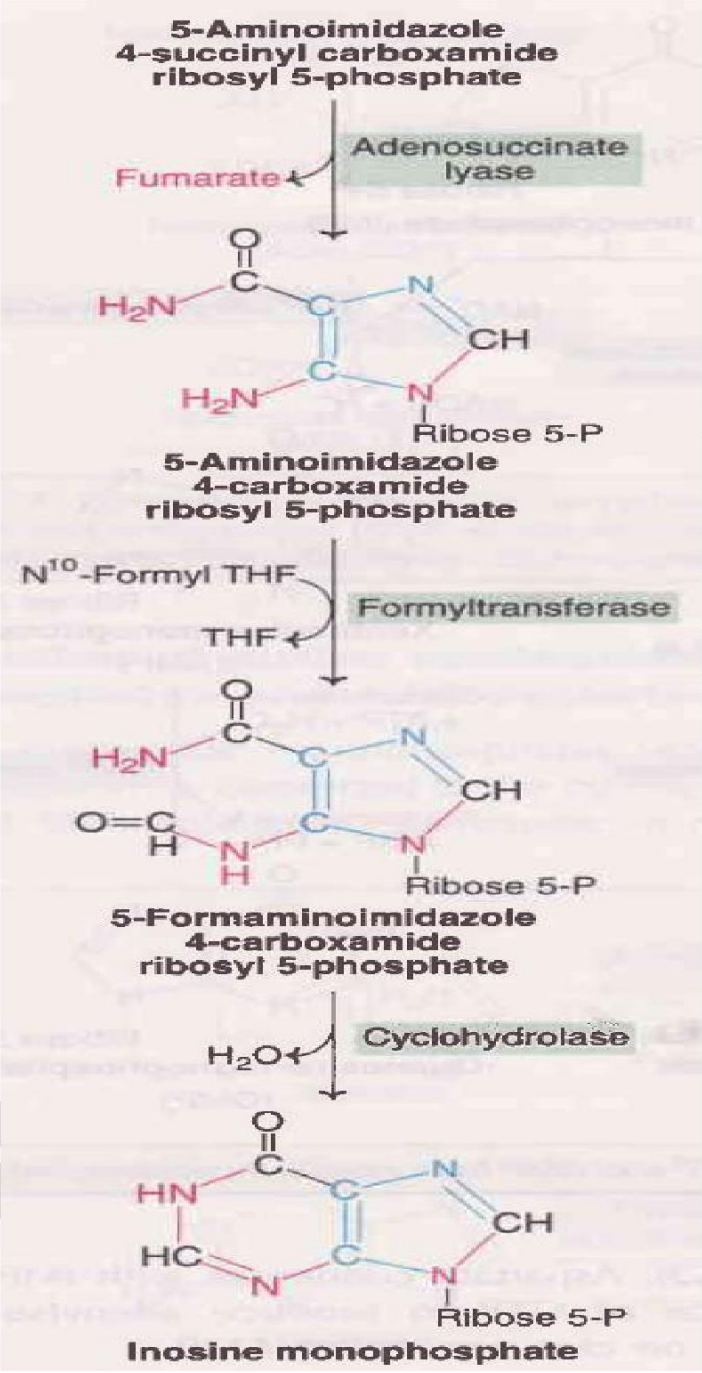
Since biosynthesis of IMP consumes glycine, glutamine, tetrahydrofolate derivatives, aspartate, and ATP,it is advantageous to regulate purine biosynthesis. The major determinant of the rate of de novo purine nucleotide biosynthesis is the concentration of PRPP, whose pool size depends on its rates of 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





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

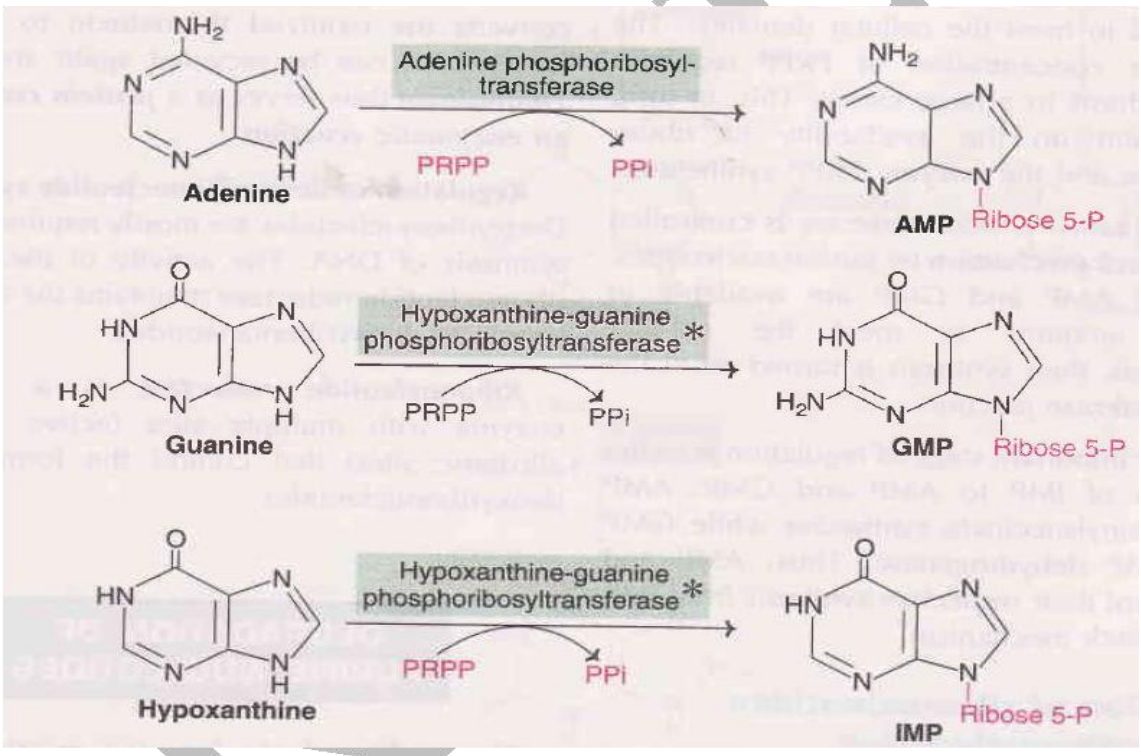
The free purines (adenine, guanine and hypoxanthine) are formed in the normal turnover of nucleic acids (particularly RNA), and also obtained from the dietary sources. The purines can be directly converted to the corresponding nucleotides, and this process is known as 'salvage pathway'. Conversion of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

$$\mathbf{Pu + PR{-}PP \rightarrow PRP + PPi}$$

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

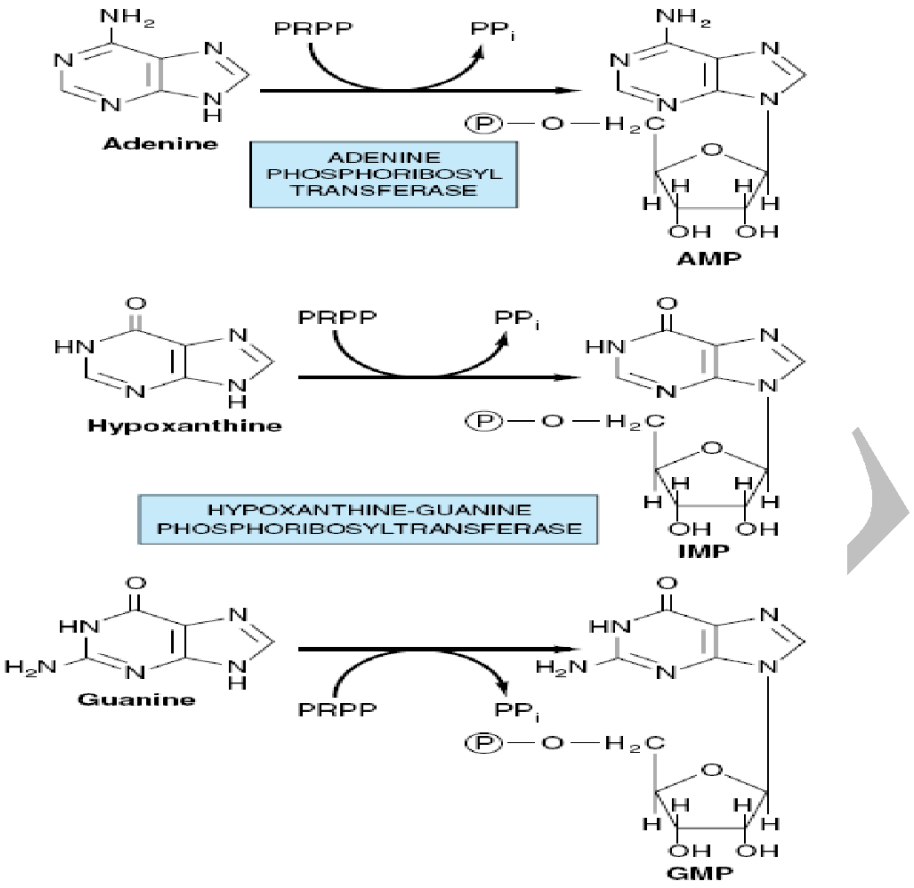
Five of the first six enzyme activities of pyrimidine biosynthesis reside on multifunctional polypeptides. One such polypeptide catalyzes the first three reactions and ensures efficient channeling of carbamoyl phosphate to pyrimidine biosynthesis. A second bifunctional enzyme catalyzes reactions 5 and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

Deoxyribonucleosides of Uracil & Cytosine

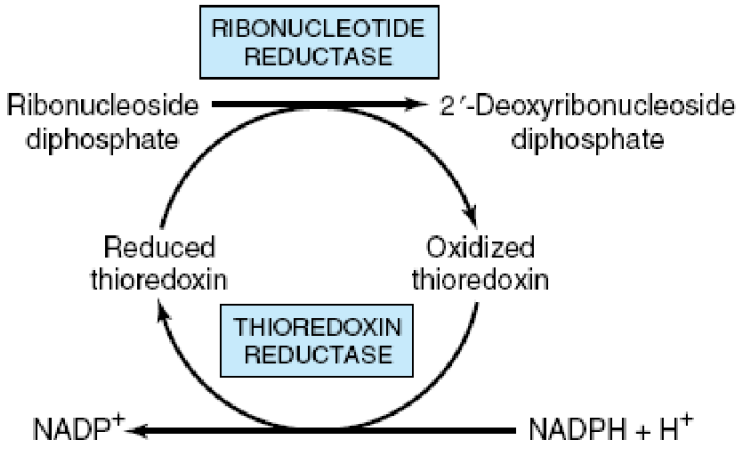
While mammalian cells reutilize few free pyrimidines, ―salvage reactions‖ convert the ribonucleosides uridine and cytidine and the deoxyribonucleosides thymidine and deoxycytidine to their respective nucleotides. ATP dependent phosphoryl transferases (kinases) catalyze the phosphorylation of the nucleoside 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.



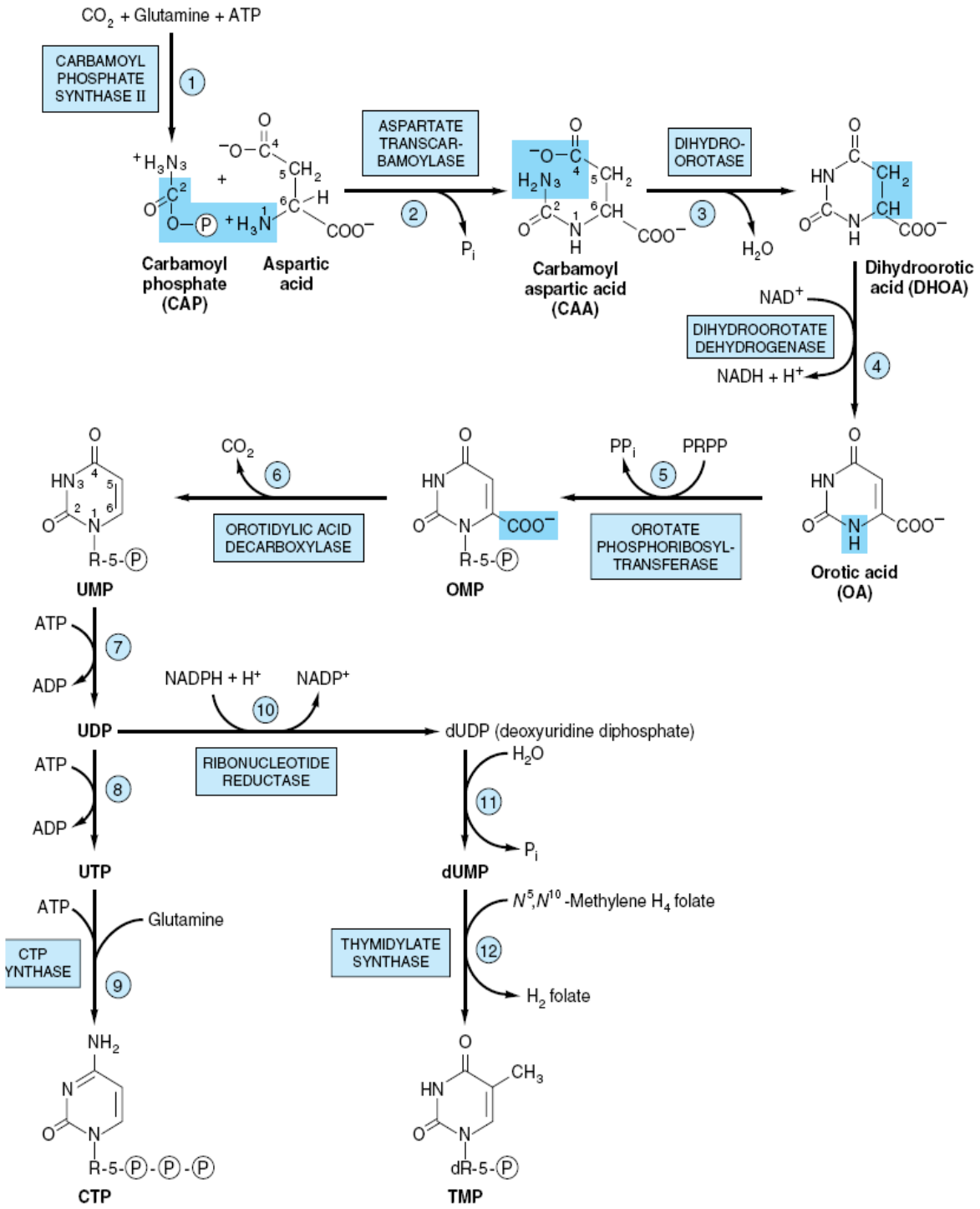
Reduction of ribonucleoside diphosphates to 2′-deoxyribonucleoside diphosphates

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.


CO2 + Glutamine + ATP
CARBAMOYL PHOSPHATE SYNTHASE II
1
Carbamoyl phosphate (CAP)
Aspartic acid
ASPARTATE TRANSCAR-BAMOYLASE
2
Pi
Carbamoyl aspartic acid (CAA)
DIHYDRO-OROTASE
3
H2O
Dihydroorotic acid (DHOA)
NAD+
DIHYDROOROTATE DEHYDROGENASE
NADH + H+
4
Orotic acid (OA)
PPi
PRPP
5
OROTATE PHOSPHORIBOSYL-TRANSFERASE
OMP
CO2
6
OROTIDYLIC ACID DECARBOXYLASE
UMP
ATP
ADP
7
UDP
NADPH + H+
NADP+
10
RIBONUCLEOTIDE REDUCTASE
dUDP (deoxyuridine diphosphate)
H2O
11
Pi
dUMP
ATP
ADP
8
UTP
ATP
Glutamine
CTP YNTHASE
9
CTP
N5,N10-Methylene H4 folate
THYMIDYLATE SYNTHASE
12
H2 folate
TMP

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.

❖ Glutamine transfers its amido nitrogen to CO_2 to produce carbamoyl phosphate. This reaction is ATP-dependent and is catalyzed by cytosomal enzyme carbamoyl phosphate synthetase II (CPS II). CPS II is activated by ATP and PRPP and inhibited by UTP. Carbamoyl phosphate synthetase (CPS I) is a mitochondrial enzyme which synthesizes carbamoyl phosphate from ammonia and CO_2.

❖ 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^{10}-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

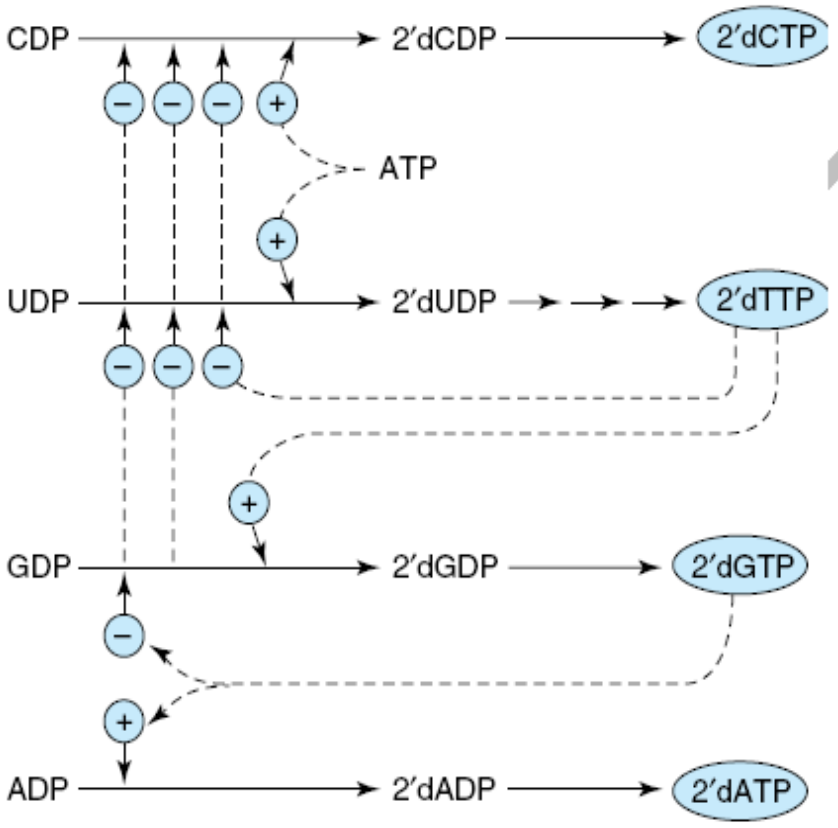
Gene Expression & Enzyme Activity Both Are Regulated

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.



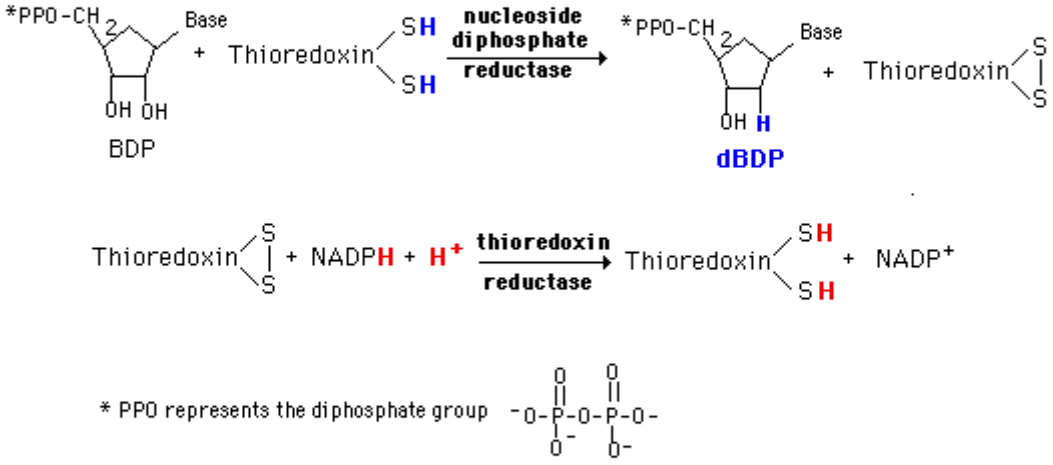
Regulation of the reduction of purine and pyrimidine ribonucleotides to their respective 2′-deoxyribonucleotides. Solid lines represent chemical flow. Broken lines show negative (–) or positive (+) feedback regulation.

PRPP

Formation of Deoxyribonucleotides

De novo synthesis and most of the salvage pathways involve the ribonucleotides. (Exception is the small amount of salvage of thymine indicated above.) Deoxyribonucleotides for DNA synthesis are formed from the ribonucleotide diphosphates (in mammals and *E. coli*).

A base diphosphate (BDP) is reduced at the 2' position of the ribose portion using the protein, **thioredoxin** and the enzyme **nucleoside diphosphate reductase**. Thioredoxin has two sulfhydryl groups which are oxidized to a disulfide bond during the process. In order to restore the thioredoxin to its reduced for so that it can be reused, **thioredoxin reductase and NADPH** are required.

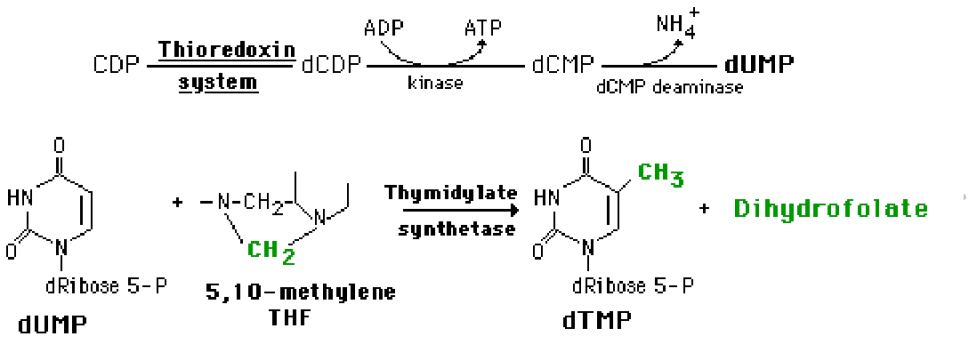


This system is very tightly controlled by a variety of allosteric effectors. dATP is a general inhibitor for all substrates and ATP an activator. Each substrate then has a specific positive effector (a BTP or dBTP). The result is a maintenance of an appropriate balance of the deoxynucleotides for DNA synthesis.

Synthesis of dTMP

DNA synthesis also requires dTMP (dTTP). This is not synthesized in the de novo pathway and salvage is not adequate to maintain the necessary amount. dTMP is generated from dUMP using the folate-dependent one-carbon pool.

Since the nucleoside diphosphate reductase is not very active toward UDP, CDP is reduced to dCDP which is converted to dCMP. This is then deaminated to form dUMP. In the presence of **5,10-Methylene tetrahydrofolate** and the enzyme **thymidylate synthetase**, the carbon group is both transferred to the pyrimidine ring and further reduced to a methyl group. The other product is **dihydrofolate** which is subsequently reduced to the tetrahydrofolate by dihydrofolate reductase.

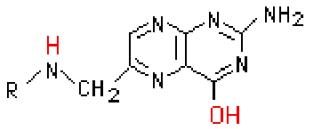


Dihydrofolate must be subsequently reduced to the tetrahydro form.

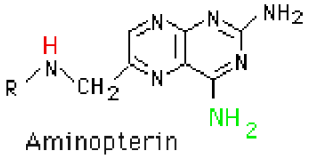
Chemotherapeutic Agents

Thymidylate synthetase is particularly sensitive to availability of the folate one-carbon pool. Some of the cancer chemotherapeutic agents interfere with this process as well as with the steps in purine nucleotide synthesis involving the pool.

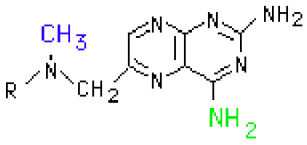
Cancer chemotherapeutic agents like **methotrexate** (4-amino, 10-methyl folic acid) and **aminopterin** (4-amino, folic acid) are structural analogs of folic acid and inhibit dihydrofolate reductase. This interferes with maintenance of the folate pool and thus of de novo synthesis of purine nucleotides and of dTMP synthesis. Such agents are highly toxic and administered under careful control.



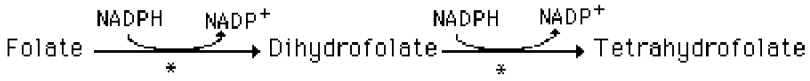
Folate



Aminopterin



Methotrexate



* Aminopterin and Methotrexate are inhibitors of dihydrofolate reductase

METABOLIC PROFILE OF MAJOR ORGANS

The metabolic patterns of the brain, muscle, adipose tissue, kidney, and liver are strikingly different. Let us consider how these organs differ in their use of fuels to meet their energy needs:

1. **Brain**. Glucose is virtually the sole fuel for the human brain, except during prolonged starvation. The brain lacks fuel stores and hence requires a continuous supply of glucose. It consumes about 120 g daily, which corresponds to an energy input of about 420 kcal (1760 kJ), accounting for some 60% of the utilization of glucose by the whole body in the resting state.
Much of the energy, estimates suggest from 60% to 70%, is used to power transport mechanisms that maintain the Na^+-K^+ membrane potential required for the transmission of the nerve impulses. The brain must also synthesize neurotransmitters and their receptors to propagate nerve impulses. Overall, glucose metabolism remains unchanged during mental activity, although local increases are detected when a subject performs certain tasks.

Glucose is transported into brain cells by the glucose transporter GLUT3. This transporter has a low value of K_M for glucose (1.6 mM), which means that it is saturated under most conditions. Thus, the brain is usually provided with a constant supply of glucose. Noninvasive ^{13}C nuclear magnetic resonance measurements have shown that the concentration of glucose in the brain is about 1 mM when the plasma level is 4.7 mM (84.7 mg/dl), a normal value. Glycolysis slows down when the glucose level approaches the K_M value of hexokinase (~50 μM), the enzyme that traps glucose in the cell (Section 16.1.1). This danger point is reached when the plasma-glucose level drops below about 2.2 mM (39.6 mg/dl) and thus approaches the K_M value of GLUT3.

Fatty acids do not serve as fuel for the brain, because they are bound to albumin in plasma and so do not traverse the blood-brain barrier. In starvation, ketone bodies generated by the liver partly replace glucose as fuel for the brain.

2. **Muscle**. The major fuels for muscle are glucose, fatty acids, and ketone bodies. Muscle differs from the brain in having a large store of glycogen (1200 kcal, or 5000 kJ). In fact, about three-fourths of all the glycogen in the body is stored in muscle. This glycogen is readily converted into glucose 6-phosphate for use within muscle cells. Muscle, like the brain, lacks glucose 6-phosphatase, and so it does not export glucose. Rather, muscle retains glucose, its preferred fuel for bursts of activity.

In actively contracting skeletal muscle, the rate of glycolysis far exceeds that of the citric acid cycle, and much of the pyruvate formed is reduced to lactate, some of which flows to the liver, where it is converted into glucose.

Metabolic Interchanges between Muscle and Liver.

These interchanges, known as the Cori cycle, shift part of the metabolic burden of muscle to the liver. In addition, a large amount of alanine is formed in active muscle by the transamination of pyruvate. Alanine, like lactate, can be converted into glucose by the liver. Why does the muscle release alanine? Muscle can absorb and transaminate branched-chain amino acids; however, it cannot form urea. Consequently, the nitrogen is released into the blood as alanine. The liver absorbs the alanine, removes the nitrogen for disposal as urea, and processes the pyruvate to glucose or fatty acids. The metabolic pattern of resting muscle is quite different. In resting muscle, fatty acids are the major fuel, meeting 85% of the energy needs.

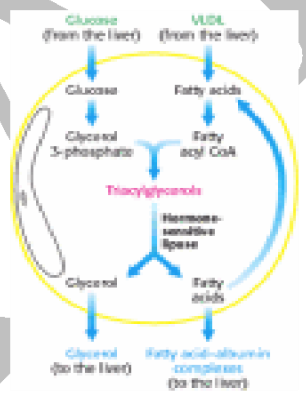
Unlike skeletal muscle, heart muscle functions almost exclusively aerobically, as evidenced by the density of mitochondria in heart muscle. Moreover, the heart has virtually no glycogen reserves. Fatty acids are the heart's main source of fuel, although ketone bodies as well as lactate can serve as fuel for heart muscle. In fact, heart muscle consumes acetoacetate in preference to glucose.

3**.Adipose tissue**. The triacylglycerols stored in adipose tissue are an enormous reservoir of metabolic fuel.

Fuel reserves in a typical 70-kg man.

In a typical 70-kg man, the 15 kg of triacylglycerols have an energy content of 135,000 kcal (565,000 kJ). Adipose tissue is specialized for the esterification of fatty acids and for their release from triacylglycerols. In human beings, the liver is the major site of fatty acid synthesis. Recall that these fatty acids are esterified in the liver to glycerol phosphate to form triacylglycerol and are transported to the adipose tissue in lipoprotein particles, such as very low density lipoproteins. Triacylglycerols are not taken up by adipocytes; rather, they are first hydrolyzed by an extracellular lipoprotein lipase for uptake. This lipase is stimulated by processes initiated by insulin. After the fatty acids enter the cell, the principal task of adipose tissue is to activate these fatty acids and transfer the resulting CoA derivatives to glycerol in the form of glycerol 3-phosphate. This essential intermediate in lipid biosynthesis comes from the reduction of the glycolytic intermediate dihydroxyacetone phosphate. Thus, adipose cells need glucose for the synthesis of triacylglycerols.

Synthesis and Degradation of Triacylglycerols by Adipose Tissue. Fatty acids are delivered to adipose cells in the form of triacylglycerols contained in very low density lipoproteins (VLDLs).



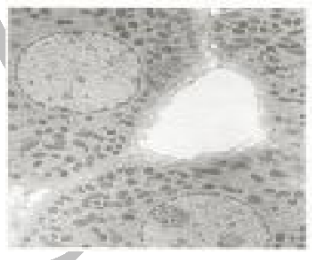
Triacylglycerols are hydrolyzed to fatty acids and glycerol by intracellular lipases. The release of the first fatty acid from a triacylglycerol, the rate-limiting step, is catalyzed by a hormone-sensitive lipase that is reversibly phosphorylated. The hormone epinephrine stimulates the formation of cyclic AMP, the intracellular messenger in the amplifying cascade, which activates a protein kinase—a recurring theme in hormone action. Triacylglycerols in adipose cells are continually being hydrolyzed and resynthesized. Glycerol derived from their hydrolysis is exported to the liver. Most of the fatty acids formed on hydrolysis are reesterified if glycerol 3-phosphate is abundant. In contrast, they are released into the plasma if glycerol 3-phosphate is

scarce because of a paucity of glucose. Thus, the glucose level inside adipose cells is a major factor in determining whether fatty acids are released into the blood.

4.**The kidney**. The major purpose of the kidney is to produce urine, which serves as a vehicle for excreting metabolic waste products and for maintaining the osmolarity of the body fluids. The blood plasma is filtered nearly 60 times each day in the renal tubules. Most of the material filtered out of the blood is reabsorbed; so only 1 to 2 liters of urine is produced. Water-soluble materials in the plasma, such as glucose, and water itself are reabsorbed to prevent wasteful loss. The kidneys require large amounts of energy to accomplish the reabsorption. Although constituting only 0.5% of body mass, kidneys consume 10% of the oxygen used in cellular respiration. Much of the glucose that is reabsorbed is carried into the kidney cells by the sodium-glucose cotransporter. Recall that this transporter is powered by the Na^{+}-K^{+} gradient, which is itself maintained by the Na^{+}-K^{+} ATPase. During starvation, the kidney becomes an important site of gluconeogenesis and may contribute as much as half of the blood glucose.

5.Liver. The metabolic activities of the liver are essential for providing fuel to the brain, muscle, and other peripheral organs. Indeed, the liver, which can be from 2% to 4% of body weight, is an organism's metabolic hub.

Electron Micrograph of Liver Cells. The liver plays an essential role in the integration of metabolism. [Courtesy of Dr. Ann Hubbard.]



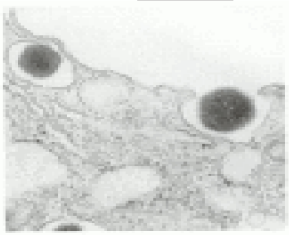
Most compounds absorbed by the intestine first pass through the liver, which is thus able to regulate the level of many metabolites in the blood.

Let us first consider how the liver metabolizes carbohydrates. The liver removes two-thirds of the glucose from the blood and all of the remaining monosaccharides. Some glucose is left in the blood for use by other tissues. The absorbed glucose is converted into glucose 6-phosphate by hexokinase and the liver-specific glucokinase. Glucose 6-phosphate, as already stated, has a variety of fates, although the liver uses little of it to meet its own energy needs. Much of the glucose 6-phosphate is converted into glycogen. As much as 400 kcal (1700 kJ) can be stored in this form in the liver. Excess glucose 6-phosphate is metabolized to acetyl CoA, which is used to form fatty acids, cholesterol, and bile salts. The pentose phosphate pathway, another means of

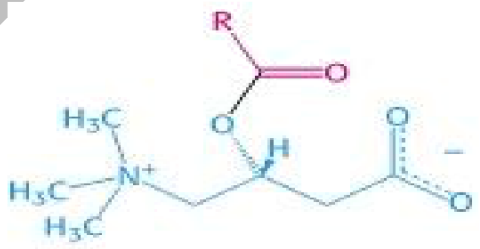
processing glucose 6-phosphate, supplies the NADPH for these reductive biosyntheses. The liver can produce glucose for release into the blood by breaking down its store of glycogen and by carrying out gluconeogenesis. The main precursors for gluconeogenesis are lactate and alanine from muscle, glycerol from adipose tissue, and glucogenic amino acids from the diet.

The liver also plays a central role in the regulation of lipid metabolism. When fuels are abundant, fatty acids derived from the diet or synthesized by the liver are esterified and secreted into the blood in the form of very low density lipoprotein.

Insulin Secretion. The electron micrograph shows the release of insulin from a pancreatic β cell. One secretory granule is on the verge of fusing with the plasma membrane and releasing insulin into the extracellular space, and the other has already.



However, in the fasting state, the liver converts fatty acids into ketone bodies. How is the fate of liver fatty acids determined? The selection is made according to whether the fatty acids enter the mitochondrial matrix. Recall that long-chain fatty acids traverse the inner mitochondrial membrane only if they are esterified to carnitine. Carnitine acyltransferase I (also known as carnitine palmitoyl transferase I), which catalyzes the formation of acyl carnitine, is inhibited by malonyl CoA, the committed intermediate in the synthesis of fatty acids. Thus, when malonyl CoA is abundant, long-chain fatty acids are prevented from entering the mitochondrial matrix, the compartment of β-oxidation and ketone-body formation. Instead, fatty acids are exported to adipose tissue for incorporation into triacylglycerols. In contrast, the level of malonyl CoA is low when fuels are scarce. Under these conditions, fatty acids liberated from adipose tissues enter the mitochondrial matrix for conversion into ketone bodies.



Fatty acyl carnitine

The liver also plays an essential role in dietary amino acid metabolism. The liver absorbs the majority of amino acids, leaving some in the blood for peripheral tissues. The priority use of amino acids is for protein synthesis rather than catabolism. By what means are amino acids directed to protein synthesis in preference to use as a fuel? The K_M value for the aminoacyl-tRNA synthetases is lower than that of the enzymes taking part in amino acid catabolism. Thus, amino acids are used to synthesize aminoacyl-tRNAs before they are catabolized. When catabolism does take place, the first step is the removal of nitrogen, which is subsequently processed to urea. The liver secretes from 20 to 30 g of urea a day. The α-ketoacids are then used for gluconeogenesis or fatty acid synthesis. Interestingly, the liver cannot remove nitrogen from the branch-chain amino acids (leucine, isoleucine, and valine). Transamination takes place in the muscle.

How does the liver meet its own energy needs? α-Ketoacids derived from the degradation of amino acids are the liver's own fuel. In fact, the main role of glycolysis in the liver is to form building blocks for biosyntheses. Furthermore, the liver cannot use acetoacetate as a fuel, because it has little of the transferase needed for acetoacetate's activation to acetyl CoA. Thus, the liver eschews the fuels that it exports to muscle and the brain.

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‖.

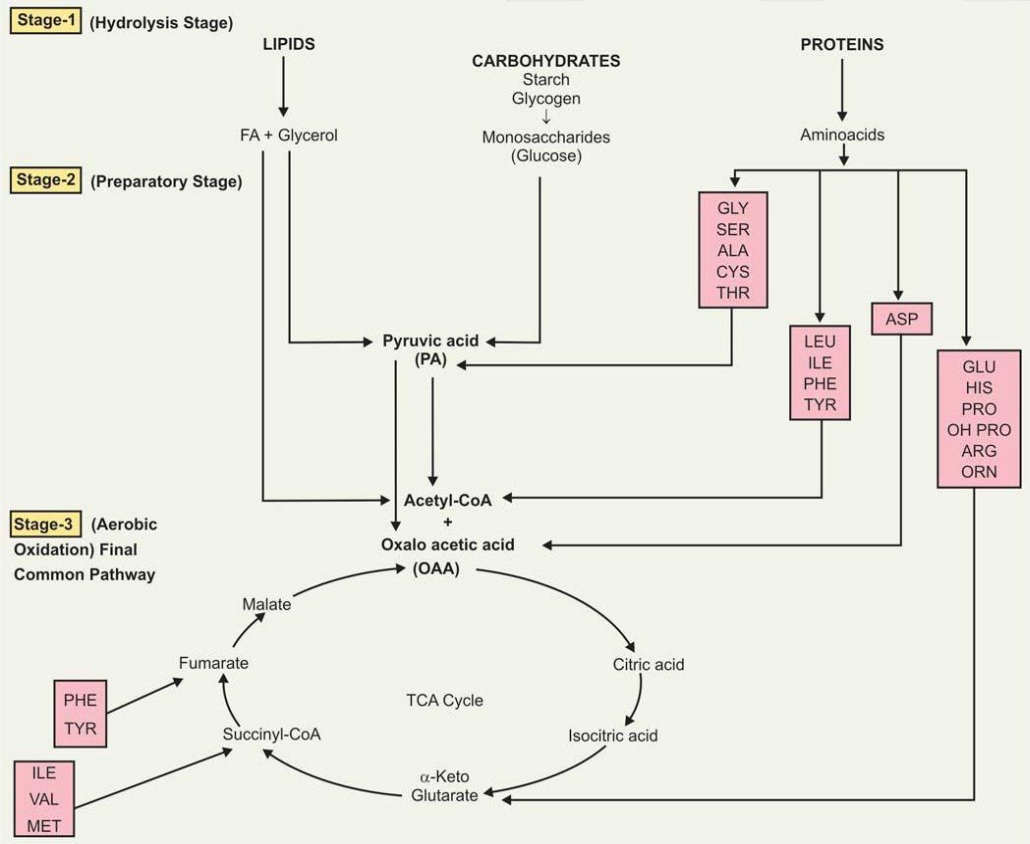
During the second stage (glycolysis, β-oxidation, etc.) relatively small amount of energy is produced and this is stored as ATP.

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

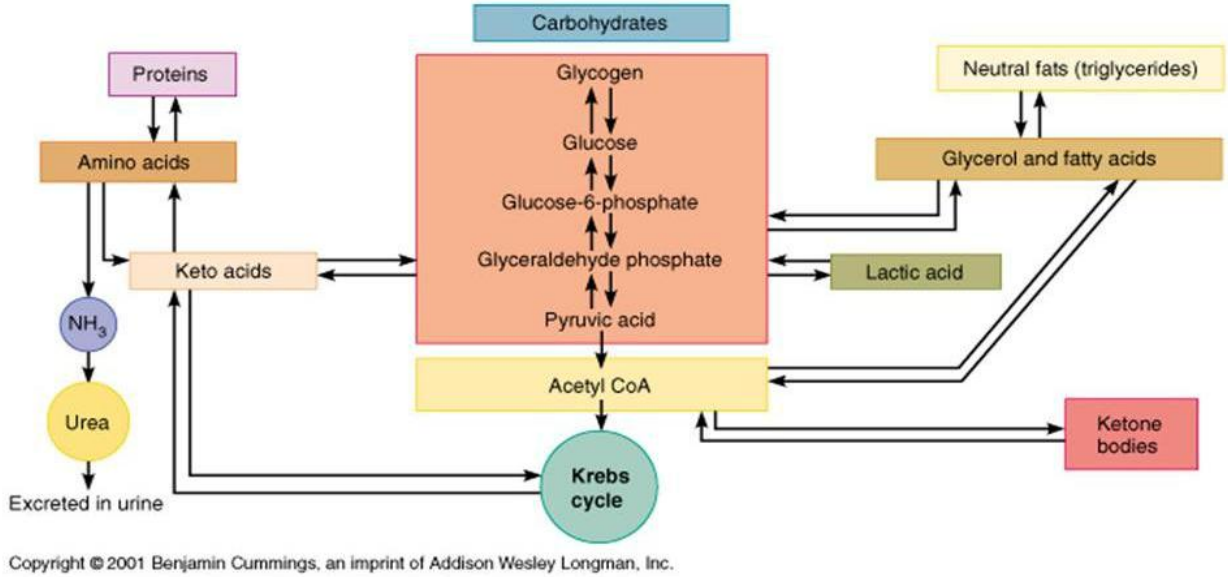
will also be channelised to HMP-shunt leading to increased formation of NADPH which will participate in reductive synthesis, like FA synthesis which will be increased. The converse happens with low ATP and high AMP levels.

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.

Interconversion of carbohydrate, lipid and protein



SCID

Right after they're born, babies are protected from infections by immunity transmitted to them by their mothers. Within the next few months, though, their immune systems develop and begin to assume responsibility for fighting off infections. But sometimes, babies have immune deficiencies and they don't have the ability to fight off routine infections on their own.

The symptoms of immune deficiency depend on what part of the immune system is affected and can range from mild to life-threatening. One example of a life-threatening immune deficiency is severe combined immunodeficiency (SCID).

SCIDSCID, an uncommon disease, can be successfully treated if it's identified early. Otherwise, it can be fatal within the first year of life.

What Is SCID?

SCID is actually a group of inherited disorders that cause severe abnormalities of the immune system. These disorders lead to reduced or malfunctioning T- and B-lymphocytes, the specialized white blood cells made in the bone marrow to fight infection. When the immune system doesn't function properly, it can be difficult or impossible for it to battle viruses, bacteria, and fungi that cause infections.

SCID is called "combined" immunodeficiency because it affects the function of two kinds of infection-fighting cells where other immune system diseases involve only one. There are several forms of SCID. The most common type is caused by a problem in a gene found on the X chromosome and affects only males. Females may be carriers of the condition, but because they also inherit a normal X chromosome, their immune systems usually can fight infections normally. Males, on the other hand, only have one X chromosome.

Another form is caused by a deficiency of the enzyme adenosine deaminase (ADA). Other cases of SCID are caused by a variety of other genetic defects.

Diagnosing SCID

Classic signs of SCID include an increased susceptibility to infection and failure to thrive as a result of infections. A baby with SCID may have recurrent bacterial, viral, or fungal infections that are much more serious and less responsive to treatment than would normally be expected. These can include ear infections (acute otitis media), sinus infections (sinusitis), oral thrush (a type of yeast infection in the mouth), skin infections, meningitis, and pneumonia. Infants with SCID may also have chronic diarrhea. If a child has these symptoms, a doctor will test for SCID or other types of immune deficiency.

Parents who have a child with SCID or a family history of immunodeficiency might want to consider genetic counseling and early blood testing, since early diagnosis can lead to prompt treatment and improve the chances of a good outcome. It may also be possible to test a high-risk baby for the disease before birth if the genetic mutation causing SCID in a family is known. Babies born with SCID can have a healthy immune system if they are treated early in life.

Most children without a known family history of the disease are not diagnosed until 6 months of age or older.

Treating SCID

When a child is diagnosed with SCID, a referral typically is made to a doctor who specializes in treating immune deficiencies — usually a pediatric immunologist or pediatric infectious disease expert.

It's important to prevent infections in kids with SCID, so your doctor may prescribe antibiotics to prevent infection and advise keeping the child away from crowds and sick people.

Children with SCID should not be immunized with live viruses — like the chickenpox (varicella) or measles, mumps, and rubella (MMR) vaccines — because they lack the normal defense of antibodies to the viruses. Introducing a virus, even a weakened vaccine virus, can be dangerous.

Doctors may also administer an infusion of intravenous immune globulin (IVIG) to help the body fight infection.

The most effective treatment for SCID is a **stem cell transplant**. This is when stem cells — cells found primarily in the bone marrow from which all types of blood cells develop — are introduced into the body in the hopes that the new cells will rebuild the immune system.

To provide the best chances for success, a transplant is usually done using the bone marrow of a sibling. However, a parent's marrow might also be acceptable. Some children do not have family members who are suitable donors — in such cases, doctors may use stem cells from an unrelated donor. The likelihood of a good outcome also is higher if the transplant is done early, within the first few months of life, if possible.

Some SCID patients require chemotherapy before their transplant. Chemotherapy will destroy cells in the bone marrow to make room for the donated cells and help prevent the child's immune cells from attacking the donated cells. Other kids with SCID may not need such treatment, especially if they have very few immune cells to start with. The use of pre-transplant chemotherapy depends on the severity of the immune deficiency, the type of SCID, the donor used, and the transplant center.

In cases of SCID caused by a missing enzyme, the enzyme can be replaced via a weekly injection. This is not a cure and these children must receive the injections for the rest of their lives.

Another treatment approach currently being studied is **gene therapy**. This involves removing cells from a child with SCID and inserting healthy genes into them, then transplanting them back into the child. When they find their way to the bone marrow, they can start to produce healthy immune cells. Gene therapy has been successful for some patients with certain types of SCID, but a few children treated with it developed complications, so it has not yet become routine treatment. New trials of gene therapy are ongoing.

Gout

What is gout?

Gout is a kind of arthritis. It can cause an attack of sudden burning pain, stiffness, and swelling in a joint, usually a big toe. These attacks can happen over and over unless gout is treated. Over time, they can harm your joints, tendons, and other tissues. Gout is most common in men.

What causes gout?

Gout is caused by too much uric acid in the blood. Most of the time, having too much uric acid is not harmful. Many people with high levels in their blood never get gout. But when uric acid levels in the blood are too high, the uric acid may form hard crystals in your joints.

Your chances of getting gout are higher if you are overweight, drink too much alcohol, or eat too much meat and fish that are high in chemicals called purines. Some medicines, such as water pills (diuretics), can also bring on gout.

What are the symptoms?

The most common sign of gout is a nighttime attack of swelling, tenderness, redness, and sharp pain in your big toe. You can also get gout attacks in your foot, ankle, or knees. The attacks can last a few days or many weeks before the pain goes away. Another attack may not happen for months or years.

See your doctor even if your pain from gout is gone. The buildup of uric acid that led to your gout attack can still harm your joints.

How is gout diagnosed?

Your doctor will ask questions about your symptoms and do a physical exam. Your doctor may also take a sample of fluid from your joint to look for uric acid crystals. This is the best way to test for gout. Your doctor may also do a blood test to measure the amount of uric acid in your blood.

How is it treated?

To stop a gout attack, your doctor can give you a shot of corticosteroids, or prescribe a large daily dose of one or more medicines. The doses will get smaller as your symptoms go away. Relief from a gout attack often begins within 24 hours if you start treatment right away.

To ease the pain during a gout attack, rest the joint that hurts. Taking ibuprofen or another anti-inflammatory medicine can also help you feel better. But don't take aspirin. It can make gout worse by raising the uric acid level in the blood.

To prevent future attacks, your doctor can prescribe a medicine to reduce uric acid buildup in your blood. If your doctor prescribes medicine to lower your uric acid levels, be sure to take it as directed. Most people continue to take this medicine for the rest of their lives.

Paying attention to what you eat may help you manage your gout. Eat moderate amounts of a healthy mix of foods to control your weight and get the nutrients you need. Avoid regular daily intake of meat, seafood, and alcohol (especially beer). Drink plenty of water and other fluids.

POSSIBLE QUESTIONS

2 mark questions

1. Sketch the regulation of ribonucleotide reductase
2. 14) Give the clinical conditions of gout
3. Add short notes on metabolic profile of muscle
4. Sketch the regulation of purine synthesis
5. Explain the clinical condition SCID
6. Add notes on metabolic profile of liver
7. Sketch the regulation of pyrimidine synthesis
8. Add short notes on SCID
9. Give ahort note on the molecules involved with the construction of purine ring
10. Differentiate the carbamoyl phosphate synthase I and carbamoyl phosphate synthase II

Essay type questions (6Marks)

1. Discuss in detail about the de novo synthesis and regulation of purines
2. Describe the synthesis and regulation of pyrimidine nucleotides
3. Discuss the synthesis and regulation of deoxy ribo nucleotides
4. How deoxy ribo nucleotides are controlled by inhibitors?
5. Explain the metabolic profile of liver and adipose tissue
6. Describe the tissue specific metabolism of brain and muscle
7. Explain the pathophysiology and treatment of gout and SCID

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KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I MSc BIOCHEMISTRY-Second Semester

REGULATION OF METABOLIC PATHWAYS (18BCP201)

UNIT-V **MULTIPLE CHOICE QUESTIONS**

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	In purine biosynthesis ,α-d-ribulose-5-phosphate to PRPP is catalyzed by	PRPP transferase	PRPP amidotransferase	PRPP synthase	none of the above	PRPP synthase
2	The compound that inhibits thymidylate synthase is _____	hydroxyurea	6-mercaptopurine	methotrexate	trimethoprine	methotrexate
3	β-uriedopropionic acid is an intermediate in the _____	synthesis of purine	degradation of purine	synthesis of pyrimidine	degradation of pyrimidine	degradation of pyrimidine
4	ATP+CO2+glutamine→ CAP.this reaction is catalyzed by _____	carbanyl phosphate synthaseII	aspartate transcarbamylase	ortate phosphoribosyl transferase	CTP synthase	carbanyl phosphate synthaseII
5	One of the following is the example for purine biosynthesis inhibitor _____	arsinate	fluroacetate	vitamin B12	azaserine	azaserine
6	The reduction of NDPs to NTPs is catalyzed by _____	ribonucleotidedehydrogenase	ribonucleotide reductase complex	PRPP synthase	adenine phosphoryl transferase	ribonucleotide reductase complex
7	The conversion IMP into GMP requires the following except _____	NAD+	glutamine	ATP	GTP	GTP
8	The mitochondrial enzymes of pyrimidine nucleotide biosyunthesis is	carbamoylphosphate synthasen II	aspartate trans carbamoylase	dihydroorotate dehydrogenase	dihydroorotase	carbamoylphosphate synthasen II
9	Reduction of ribonucleotides(RN) to deoxyRN in mammals requires the following except	ribonucleotide reductase complex	vit-B12	thioredoxin	NADPH	vit-B12
10	compound that inhibits xanthine oxidase_____	azaserine	mycopholic acid	diazanoleucine	allopurinol	allopurinol
11	The enzyme sensitive to methotrexate in the pyrimidine synthesis is _____	dihydroorotate dehydrogenase	thymidylate synthase	ribonucleotide redctase	CTP synthase	thymidylate synthase

12	The end product of purine catabolism in humans _____	ammonia	uric acid	allatoin	urea	uric acid
13	The conversion of dihydroorotic acid to orotic acid is effected by the enzyme _____	dihydro orotase	dihydro orotate dehydrogenase	orotate phosphoribosyl transferase	dihydro oratate transferase	dihydro orotate dehydrogenase
14	The feed back regulator of PRPP glutamyl amido transferase is _____	UMP	GMP	CMP	TMP	GMP
15	The committed step in purine nucleotide biosyunthesis is the formation of _____	5-phospho ribosylamine	5-phospho deoxy ribosylamine	adenosine5’ phosphate	guanosine 5’phosphate	5-phospho ribosylamine
16	The activity of riboucleoside diphosphate is allosterically regulated by _____	nucleoside triphosphates	nucleoside mono phosphates	ucleoside diphosphates	Ribose sugar	nucleoside triphosphates
17	Cyclic AMP is formed from ATP by the enzyme _____	phosphadiesterase	cyclic AMP phosphodiesterase	adenylate cyclase	xanthine oxidase	adenylate cyclase
18	NTP acts as a precursor for the polymerization _____	fatty acid	carboxylic acid	nucleic acid	amino acids	nucleic acid
19	Orotic aciduria type I is due to defect in _____	oratate phosphoribosyl transferase	orotidylate decarboxylase	aspartate trans carbamoylase	dihydro-orotase	oratate phosphoribosyl transferase
20	Carbamyl phosphate synthase activity is activated by _____	phosphoribosyl pyrophosphate	purine nucleotides	aspartic acid	thymidine triphosphate	purine nucleotides
21	Denovo synthesis of purine nucleotide takes place predominantly in _____	liver	brain	erythrocytes	polymorphonuclear leucocytes	liver
22	Synthesis of deoxy ribo nucleotide requires _____	dihydrobiopterin	thioredoxin	N5N10 methylene tetrahydrofolate	N5 formyl tetra hydrofolate	thioredoxin
23	Orotic aciduria is due to excessive function of_____	purine metabolism	pyrimidine metabolism	icotinic acid metabolism	orotic acid metabolism	orotic acid metabolism
24	Glutamine synthetase is regulated by _____	concerted feed back inhibition	cumulative feed back inhibition	sequential feed back inhibition	adenylation	adenylation

25	The binding of dATP to ribonucleotide reductase inhibits production of _____	GDP	UDP	CDP	all substrates	all substrates
26	In animals ATCase is _____	inhibited by ATP	inhibited by CTP	activated by dTTP	not a regulatory mechanism	not a regulatory mechanism
27	Cyclic AMP is formed from ATP by the enzyme _____	phosphodiesterase	cyclic AMP phosphodiesterase	adenylate cyclase	xanthine oxidase	adenylate cyclase
28	The chief end product of purine catabolism in man _____	urea	uracil	uric acid	allantoin	uric acid
29	APRTase involved in the conversion _____	ribose-5-phosphate to AMP	phosphoribosyl pyrophosphate to AMP.	hypo xanthine to AMP.	adenine to AMP	hypo xanthine to AMP.
30	End product of purine metabolism is _____	adenosine	β-alanine	butyric acid	uric acid	uric acid
31	catabolic product of pyrimidine are _____	alanine & aminoacid	β- alanionme	β-alanine & aminoisobutyric acid	buyric acid	β-alanine & aminoisobutyric acid
32	PABA analogue inhibits the enzyme_____	formyl transferase	carboxylase	cydohydrolase	adenosylosuccinate	formyl transferase
33	the drug that inhibits the reduction of dihydrofolate to tetrahydrfolate_____	vincristine	vinblastin	methotrexate	methochelate	methotrexate
34	Major determinant of overall rate of denovo purine nucleotide biosynthesis is	ribose-5- phosphate	PRPP	5-phospho ribosylamine	IMP	PRPP
35	Purine catabolism is inhibited by _____	Cytarabine	allopurinol	azathioprine	5-iodo deoxyuridine	allopurinol
36	Salvage reaction converts purine and nucleosides to _____	mono nucleotide	dinucleotide	trinucleotide	nitorgenous bases	mono nucleotide
37	Guanine to GMP is catalysed by _____	hypoxanthine guanine phosphoribosyltransferase	adenine phospho ribosyl transferase	adenosine kinasae	cytidine kinase	hypoxanthine guanine phosphoribosyltransferase

38	_____ synthesis takes place through amphibolic intermediates	Aminoacid	Purine	Fattyacid	carbohydrates	Purine
39	The compound that inhibits the conversion of IMP to XMP is _____	hydroxyurea	6-mercaptopurine	methotrexate	Mycophenolic acid	Mycophenolic acid
40	The compound that inhibits the conversion of PRPP to 5-phospho ribosylamine is	diazanorleucine	6-mercaptopurine	methotrexate	Mycophenolic acid	diazanorleucine
41	Thioredoxin is involved in	Synthesis of purine nucleotides	Synthesis of pyrimidine nucleotides	Synthesis of deoxyribo nucleotides	Synthesis of ribo nucleotides	Synthesis of deoxyribo nucleotides
42	Thioredoxin is a_____	protein cofactor	Metal cofactor	Lipid	carbohydrate	protein cofactor
43	Protein cofactor required by Thio redoxin reductase is	Thioredoxin	Thioflavin	Thioferrin	Thiol	Thioredoxin
44	Immediate reductant of NDP is	Reduced thio redoxin	oxidised thio redoxin	Thio redoxin reductase	All	Reduced thio redoxin
45	_____pathway converts purine and their nucleosides to mononucleotides	Denovo	Salvage	Both	None	Salvage
46	The sources of purine for erythrocyte is purely	Exogenous	Endogenous	Both	All	Exogenous
47	Overall rate of purine nucleotide synthesis is controlled by	PRPP	Ribose-5-phosphate	ATP	5 phospho ribosylamine	PRPP
48	Adenosine deaminase deficiency is related to _____disease.	AIDS	SCID	RA	Gout	SCID
49	Catabolic product of pyrimidine nucleotides are_____soluble	Lipid	Water	Acid	organic solvent	Water
50	Diabetes mellitus occur due to the deficiency of _____hormone	Glucagon	Insulin	Epinephrine	Glucocorticoids	Insulin
51	Diabetes mellitus is mainly charecterised by	Hypoglycemia	Hyperglycemia	Hyper lipidemia	Hypo proteinemia	Hyperglycemia
52	Untreated diabetes patients in their blood have high level of _____.	Glucose	Ketone bodies	Free fatty acid	All	Ketone bodies

53	_____enzyme is important in regulating blood glucose level after meal	Hexokinase	Phospho fructo kinase	Glucokinase	Pyruvate kinase	Glucokinase
54	Which amino acid is predominantly transported from muscle to liver during starvation	Cystein	Serine	Alanine	Threonine	Alanine
55	Among the enzyme given below which is inhibited by Glucose 6 phosphate	Hexokinase	Phospho fructo kinase	Glucokinase	Glucose 6 phosphatase	Hexokinase
56	Systemic glucose concentration is _____mmol/L	4.5-5.5	5.5-6.5	6.5-7.5	7.5-8.5	4.5-5.5
57	Normal blood glucose concentration is_____mg/dl	90-110	120-160	80-120	120-200	80-120
58	Glucagon is produced by _____ cells of pancrease	Alpha	beta	gamma	all	Alpha
59	Insulin is produced by _____ cells of pancrease	Alpha	beta	gamma	all	beta
60	In_____organ the soul source of fuel is glucose	Liver	Kidney	Brain	Spleen	Brain
61	_____increase in blood of persons with prolonged starvation	Free Fatty acid	Ketone bodies	Triacyl glycerol	all	Ketone bodies
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