ANSWER KEY

S.Y.B.Sc. Life Sciences **SEM III- Paper II**
Subject code: 79528 **Paper Code 00051981**
Date: 27th March 2019 **Marks 100**

Q. 1 Do As Directed:
Q.1.A. Fill in the blanks:

1. Cleavage of argininosuccinate forms arginine and _____.Ans. Fumaric acid

2. The surface of the lipid droplets is coated with _____.Ans. Perilipins

3. _____ is cleaved by enzyme arginase to yield Urea and Ornithine. Ans.Arginine

4. Bony fishes and amphibian tadpoles excrete ____.Ans. Ammonia

5. The glycerol released by lipase action is phosphorylated by_____ to form dihydroxyacetone phosphate.Ans. Glycerol kinase

6. β-oxidation of Palmitic acid yields __ molecules of acetyl Co-A. Ans Eight

7.γ-glutamyl phosphate donates its _____ to yield urea and ornithine. Ans. Amino group

B. Match The Columns:
Ans:

a. **iv**
b. **v**
c. **vi**
d. **vii**
e. **i**
f. **ii**
g. **iii**

C. State true or False

1. **False**
2. **true**
3. **False**
4. **False**
5. **True**
6. **False**

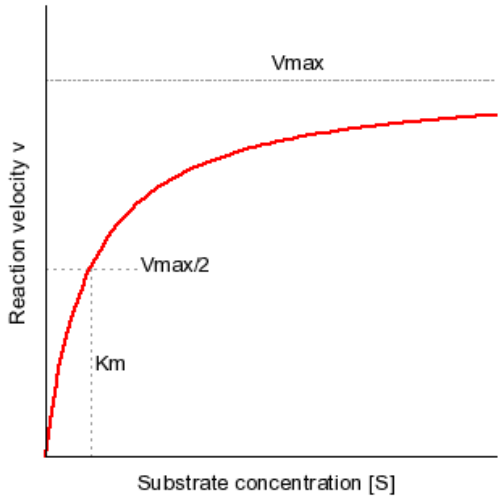
Q. 2 A) Explain any one of the following: **10**

1. Michaelis Menten Equation and effect of substrate concentration on Km and Vmax

In biochemistry, **Michaelis–Menten kinetics** is one of the best-known models of enzyme kinetics. It is named after German biochemist Leonor Michaelis and Canadian physician Maud Menten.

$$v = \frac{V_{\max}[S]}{K_M + [S]}$$

Here, $V_{\max}$ represents the maximum velocity achieved by the system, at maximum (saturating) substrate concentrations. K_M (the Michaelis constant; sometimes represented as K_S instead) is the substrate concentration at which the reaction velocity is 50% of the $V_{\max}$. [S] is the concentration of the substrate S. This is a plot of the Michaelis-Menten equation's predicted reaction velocity as a function of substrate concentration, with the significance of the kinetic parameters $V_{\max}$ and K_M graphically depicted.



$$\mathbf{Vo} = \frac{\mathbf{V_{max}[S]}}{\mathbf{Km + [S]}}$$

V0 = Initial velocity (moles/times)
[S] = substrate concentration (molar)
V_{max}= maximum velocity
K_m = substrate concentration at half V_{max}

Derivation:

An enzyme, E, binding to a substrate, S, to form a complex, ES, which in turn releases a product, P, regenerating the original enzyme. This may be represented schematically as

Applying the law of mass action, which states that the rate of a reaction is proportional to the product of the concentrations of the reactants (i.e.[E][S]), gives a system of four non-linear ordinary differential equations that define the rate of change of reactants with time .

In this mechanism, the enzyme E is a catalyst, which only facilitates the reaction, so that its total concentration, free plus combined, Km is a constant. This conservation law can also be observed by adding the first and third equations above

where K_d is the dissociation constant for the enzyme-substrate complex. Hence the velocity of the reaction – the rate at which P is formed – is

where  is the maximum reaction velocity

and V = velocity or reaction rate

= maximum velocity or maximal reaction rate

ubstrate concentration

Michaelis constant.

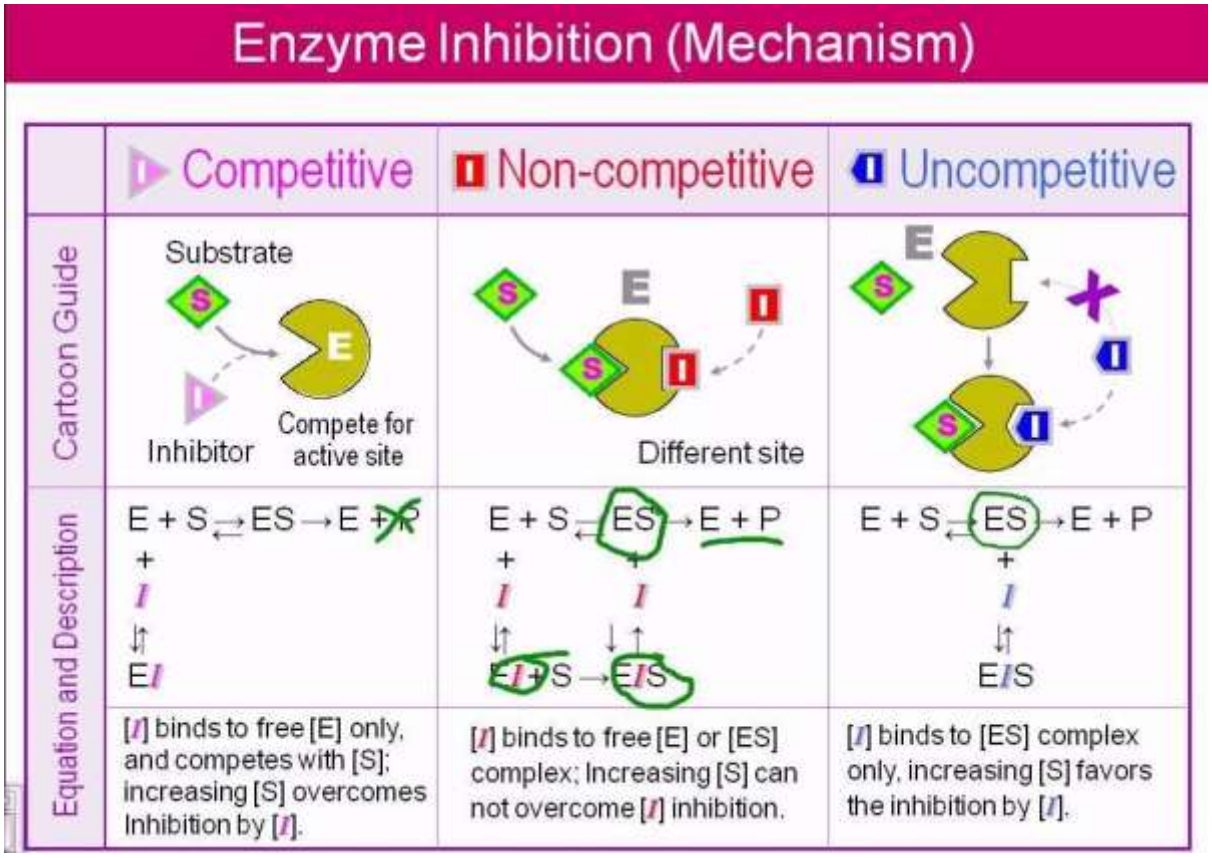
Q. 2 A)

2. Enzyme Inhibition with suitable example

Ans: -Definition of Enzyme inhibition

- Factors inhibiting enzyme action-

-Types of inhibition



Competitive inhibition, the substrate and inhibitor cannot bind to the enzyme at the same time, as shown in the figure on the right. This usually results from the inhibitor having an affinity for

the active site of an enzyme where the substrate also binds; the substrate and inhibitor *compete* for access to the enzyme's active site.

(V_{max} remains constant and the apparent K_m will increase

Uncompetitive inhibition, the inhibitor binds only to the substrate-enzyme complex. V_{max} to decreases andK_m decreases

Non-competitive inhibition, the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate.
V_{max} decreases but K_m remains the same
Any suitable example for each can be given by student.

Q. 2 B. Describe Any two of the following: 10

1. Coenzyme and Cofactors affecting enzyme action

A coenzyme is a small, organic, non-protein molecule that carries chemical groups between enzymes. It is the cofactor for the enzyme and does not form a permanent part in the enzyme's structure. Sometimes, they are called cosubstrates and are considered substrates that are loosely bound to the enzyme. In metabolism, coenzymes play a role in group-transfer reactions, such as ATP and coenzyme A, and oxidation-reduction reactions, such as NAD+ and coenzyme Q10. Coenzymes are frequently consumed and recycled. Chemical groups are added and detached continuously by an enzyme. ATP synthase enzyme phosphorylates and converts the ADP to ATP, while Kinase dephosphorylates the ATP back to ADP at continuous rates as well. Coenzyme molecules are mostly derived from vitamins. They are also commonly made from nucleotides such as adenosine triphosphate and coenzyme A.

Examples can be discussed- NADH,FADH,Co A, Vit A,C,B group
Cofactors can be **metals** or **coenzymes**, and their primary function is to assist in enzyme activity. They are able to assist in performing certain, necessary, reactions the enzyme cannot perform alone. They are divided into coenzymes and prosthetic groups. A **holoenzyme** refers to a catalytically active enzyme that consists of both **apoenzyme** (enzyme without its cofactor(s)) and cofactor. There are two groups of cofactors: metals and small organic molecules called coenzymes. Coenzymes are small organic molecules usually obtained from vitamins. **Prosthetic groups** refer to tightly bound coenzymes, while **cosubstrates** refer to loosely bound coenzymes that are released in the same way as substrates and products. Loosely bound coenzymes differ from substrates in that the same coenzymes may be used by different enzymes in order to bring about proper enzyme activity. Enzymes without their necessary cofactors are called apoenzymes, which are the inactive form of an enzyme. Cofactors with an apoenzyme are called a holoenzyme, which is the active form. Metal ions are common enzyme cofactors. Some enzymes, referred to as metalloenzymes, cannot function without a bound metal ion in the active

site. In daily nutrition, this kind of cofactor plays a role as the essential trace elements such as: iron (Fe^{3+}), manganese (Mn^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), selenium (Se^{2+}), and molybdenum (Mo^{5+}). For example, Mg^{2+} is used in glycolysis. In the first step of converting glucose to glucose 6-phosphate, before ATP is used to give ADP and one phosphate group, ATP is bound to Mg^{2+} which stabilizes the other two phosphate groups so it is easier to release only one phosphate group.

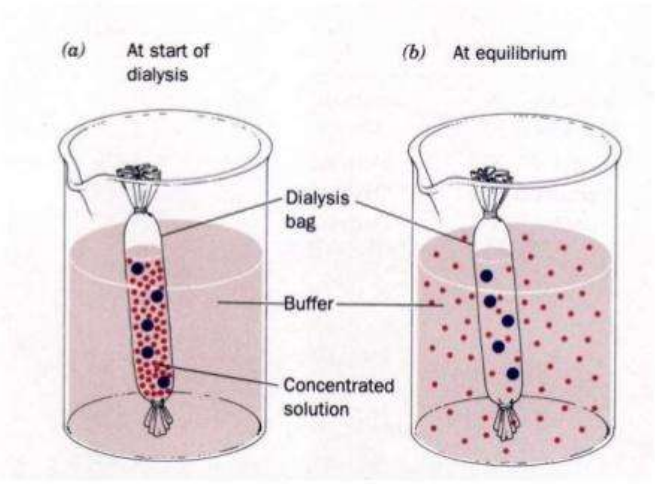
Q. 2. B

2. Dialysis as a technique in protein purification:

Dialysis is a separation technique that facilitates the removal of small, unwanted compounds from macromolecules in solution by selective and passive diffusion through a semi-permeable membrane. A sample and a buffer solution (called the dialysate, usually 200 to 500 times the volume of the sample) are placed on opposite sides of the membrane. Sample molecules that are larger than the membrane-pores are retained on the sample side of the membrane, but small molecules and buffer salts pass freely through the membrane, reducing the concentration of those molecules in the sample. Changing the dialysate buffer removes the small molecules that are no longer in the sample and allows more contaminants to diffuse into the dialysate. In this way, the concentration of small contaminants within the sample can be decreased to acceptable or negligible levels.

In dialysis a semipermeable membrane is used to separate small molecules and protein based upon their size. A dialysis bag made of a semipermeable membrane (cellulose) and has small pores. The bag is filled with a concentrated solution containing proteins. Molecules that are small enough to pass through the pores of the membrane diffuse out of the bag into the buffer solution, or dialysate. Dialysis is sometimes used to change buffers. The molecules go from an area of high concentration to low concentration. When the level of concentration is equal between the bag and the buffer, there is no more net movement of molecules. The bag is taken out and inserted into another buffer, causing the concentration to be higher in the bag relative to the buffer. This causes more diffusion of molecules. This process is repeated several times to ensure that all or most of the unwanted small molecules are removed (usually done overnight). In general, dialysis is not a means of separating proteins, but is a method used to remove small molecules such as salts. At equilibrium, larger molecules that are unable to pass through the membrane remain inside the dialysis bag while much of the small molecules have diffused out.

Figure : Use of dialysis to separate small and large molecules.



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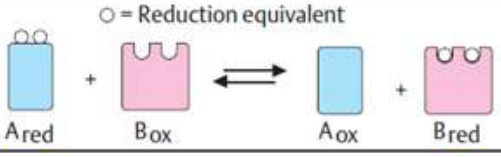
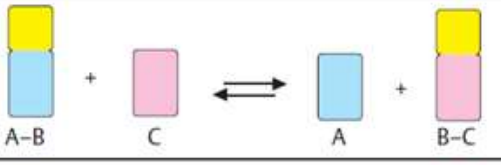
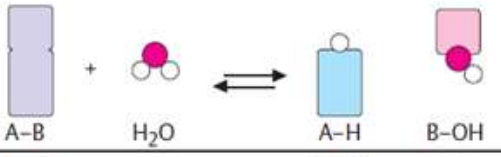
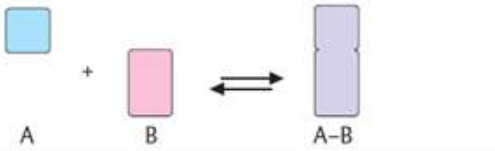
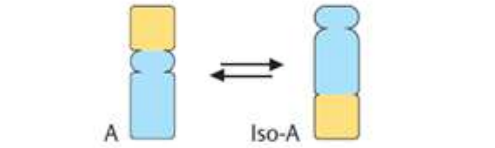
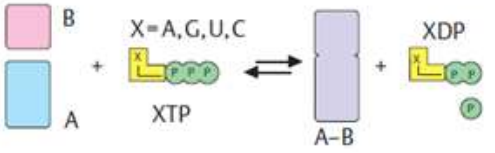
Q. 2 B

3. Any Three Classes of enzymes with their mode of action with suitable examples

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent 	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Q. 2 B

4. Allosteric Enzymes and their importance:

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector, which results in an apparent change in binding affinity at a different ligand binding site. This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept. **Allostery** plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism. Allosteric enzymes need not be oligomers as previously thought, and in fact many systems have demonstrated allostery within single enzymes. In biochemistry, allosteric regulation(or **allosteric control**) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site.

The site to which the effector binds is termed the *allosteric site*. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. 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*. Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feed forward from upstream substrates. Long-range allostery is especially important in cell signaling. Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity. The regulatory site of an allosteric protein is physically distinct from its active site. The protein catalyst (enzyme) may be part of a multi-subunit complex, and/or may transiently or permanently associate with a Cofactor (e.g. adenosine triphosphate). Catalysis of biochemical reactions is vital due to the very low reaction rates of the uncatalysed reactions. A key driver of protein evolution is the optimization of such catalytic activities via protein dynamics. Whereas enzymes without coupled domains/subunits display normal Michaelis-Menten kinetics, most allosteric enzymes have multiple coupled domains/subunits and show cooperative binding. Generally speaking, such cooperativity results in allosteric enzymes displaying a sigmoidal dependence on the concentration of their substrates in positively cooperative systems. This allows most allosteric enzymes to greatly vary catalytic output in response to small changes in effector concentration. Effector molecules, which may be the substrate itself (homotropic effectors) or some other small molecule (heterotropic effector), may cause the enzyme to become more active or less active by redistributing the ensemble between the higher affinity and lower affinity states. The binding sites for heterotropic effectors, called allosteric sites, are usually separate from the active site yet thermodynamically coupled. Allosteric enzymes thus do not show Michaelis- Menten relationship because their kinetic behaviour is greatly altered by variation in the concentration of modulators.

Allosteric enzymes play a pivotal **role** in cells because they have two functions – they not only catalyze reactions in **metabolic** pathways, but also control the rates of these pathways. Eg, The enzymatic control exerted by ATCase helps to balance the pools of purine and pyrimidine nucleotides in the cell.

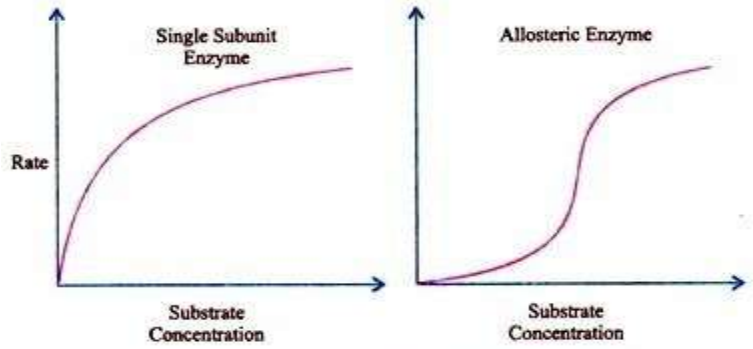


Fig. 12.15: An allosteric enzymes activity by its substrate.

Q.3 A) Answer any one of the following: (10)

1. Comment on the Amphibolic nature of Citric acid cycle with suitable examples.

Answer:

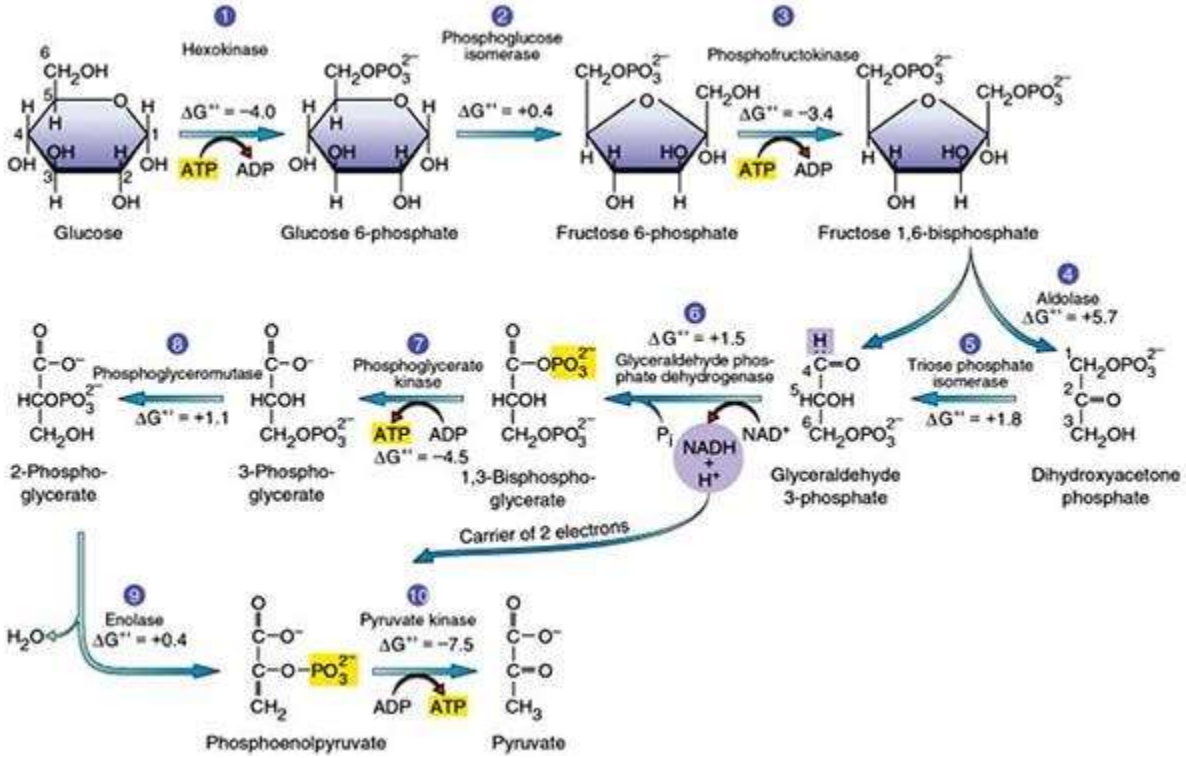
Amphibolic nature of Krebs Cycle How what we are is what we eat • In aerobic organisms, the citric acid cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes. • Since the citric acid does both synthesis (anabolic) and breakdown (catabolic) activities, it is called an amphibolic pathway • The citric acid cycle is amphibolic (i.e it is both anabolic and catabolic in its function). • It is said to be an AMPHIBOLIC pathway, because it functions in both degradative or catabolic and biosynthetic or anabolic reactions (amphi = both) A central metabolic pathway or amphibolic pathway is a set of reactions which permit the interconversion of several metabolites, and represents the end of the catabolism and the beginning of anabolism • The KREBS CYCLE or citric acid cycle is a series of reactions that degrades acetyl CoA to yield carbon dioxide, and energy, which is used to produce NADH, H+ and FADH. • The KREBS CYCLE connects the catabolic pathways that begin with the digestion and degradation of foods in stages 1 and 2 with the oxidation of substrates in stage 3 that generates most of the energy for ATP synthesis. • The citric acid cycle is the final common pathway in the oxidation of fuel molecules. In stage 3 of metabolism, citric acid is a final common catabolic intermediate in the form of acetylCoA. • This is why the citric acid cycle is called a central metabolic pathway. Anaplerosis and Cataplerosis Anaplerosis is a series of enzymatic reactions in which metabolic intermediates enter the citric acid cycle from the cytosol. Cataplerosis is the opposite, a process where intermediates leave the citric acid cycle and enter the cytosol. In muscle, anaplerosis is important for increasing citric acid throughput during periods of exercise. There is some evidence that anaplerosis is required for a glucose-induced rise in mitochondrial ATP production. Some amino acids (the building blocks of proteins) enter and leave the citric acid cycle through anaplerosis and cataplerosis. Subway Analogy Citric Acid Cycle is like a subway system: • Acetyl-CoA is like people getting on at station A • NADH is like people getting off at station B • Intermediates are like the subway cars • Anaplerosis is like adding cars to the system • Cataplerosis is like removing cars to use for spare parts Krebs Cycle is Amphibolic • Contains both catabolic and anabolic reactions. • Catabolic – Energy from oxidation of acetyl CoA is stored in reduced coenzymes. • Anabolic – Several intermediates are precursors in biosynthetic pathways. Diagram of the citric acid cycle, indicating positions at which intermediates are drawn off for use in anabolic pathways (red arrows) and points where anaplerotic reactions replenish depleted cycle intermediates (dark green arrows). Citric acid cycle intermediates are always in flux. Intermediates are brought for degradation in reactions; i.e reactions that replace cyclic intermediates. Anaplerotic pathways are a set of metabolic reactions which replenish the central metabolic pathway A B C D X Y C Z • The reaction catalyzed by pyruvate carboxylase that replenishes oxaloacetate to the TCA cycle is a good example of an anaplerotic, or “filling up,” reaction. • pyyruvate+ CO_2 + ATP +H_2O → oxaloacetate + ADP + Pi + 2 H+ • This reaction assures that there is sufficient oxaloacetate for condensation with acetyl CoA. In fact, acetyl CoA stimulates pyruvate carboxylase. • This reaction is most important, especially in liver and kidney.

ANAPLEROTIC REACTION • Pyruvate carboxylase E-biotin E-biotin~COO HCO_3 ATP ADP + P E-biotin Pyruvate Oxaloacetate Acetyl-CoA The anaplerotic, or “filling up” reaction that

connects pyruvate to the Krebs cycle • Other important anapleurotic reactions include the anapleuric reactions that are sources of succinyl CoA • Succinyl CoA is formed from fatty acids with an oddnumber of carbon atoms via propionyl CoA. • Succinyl CoA is also formed from propionyl CoA generated in the breakdown of the branched chained amino acids isoleucine, methionine, and valine • Oxidation of odd-chain fatty acids leads to production of succinyl-CoA from propionyl CoA. • Transamination and deamination of amino acids leads to production of α-ketoglutarate and oxaloacetate. Fats breakdown and feed into the TCA Cycle Breaking down fat for energy produces acetyl CoA, which feeds directly into the citric acid cycle Both fatty acids (from lipids) and amino acids (from proteins) form ACETYL CoA which enter the Citric Acid Cycle for continuation of their degradation or catabolism • Protein may serve an excellent sources of nutrient energy • Catabolism of amino acids provides: succinate, oxaloacetate, fumarate, αketoglutarate. • pyruvate (from glycolysis) • acetyl CoA stimulates pyruvate carboxylase • Proteins can also be broken down to feed into the citric acid cycle and generate energy • Under extreme starvation in animals or during senescence in plants polypeptides are cleaved to amino acids, amino group is cleaved off of amino acid forming an organic acid that can enter the citric acid cycle Degradation of amino acids that produce pyruvate or intermediates of the TCA cycle are shown at the points at which they enter the TCA. • The strategy followed by the cell in the catabolism of amino acids is to convert all twenty common amino acids into one of the following seven compounds useful in gluconeogenesis or the citric acid cycle. • acetylCoA • acetoacetyl CoA • pyruvate • oxaloacetate • fumarate • succinyl CoA • alphaketoglutarate • Most of the metabolic pathways of the body are channeled through these seven molecules. • The great importance of the two slides above resides in the fact that they highlight some of the molecules that are the major players in metabolism in the body. • These two slides indicate the position of these molecules in the citric acid cycle, and emphasize that the KREBS CYCLE is indeed a central metabolic pathway, and an amphibolic pathway; which has BOTH a catabolic and an anabolic role. . Carbon atoms from degraded amino acids are converted to the intermediates of the citric acid cycle or other pathways. Glucogenic amino acids (orange boxes) produce carbon skeletons that can form glucose, and ketogenic amino acid (green boxes) can produce ketone bodies. Protein/amino acid Catabolites feed into the TCA Cycle Oxaloacetate Acetyl-CoA Acetoacetyl-CoA α-keto glutarate Pyruvate Fumarate Succinyl-CoA Glutamate Glycine Alanine Serine Cysteine Tryptophan Tryptophan Leucine Isoleucine Leucine Lysine Phenylalanine Tyrosine Tryptophan Arginine Glutamine Histidine Proline Lysine? Isoleucine Methionine Threonine Valine Phenylalanine Tyrosine Aspartate Asparagine Anaplerosis Involving Amino Acids entering the citric acid cycle from the cytosol Pyruvate pyruvate carboxylase Anaplerosis Involving Amino Acids entering the citric acid cycle from the cytosol Glutamate Glutamine Histidine Proline Arginine + Leucine GDH Anaplerosis Involving Amino Acids entering the citric acid cycle from the cytosol Valine Isoleucine Methionine Anaplerosis Involving Amino Acids entering the citric acid cycle from the cytosol Phenylalanine Tyrosine Anaplerosis Involving Amino Acids entering the citric acid cycle from the cytosol Aspartate Asparagine

Q.3 A 2)

Q. 3 A) Answer <u>any one</u> of the following: (10)

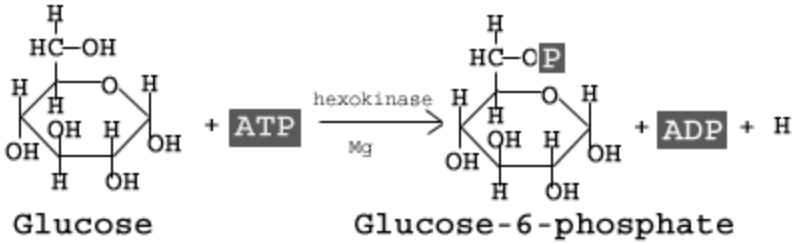


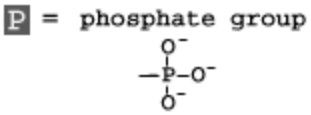
Glycolysis is the metabolic process that serves as the foundation for both aerobic and anaerobic cellular respiration. In glycolysis, glucose is converted into pyruvate. Glucose is a six-memebered ring molecule found in the blood and is usually a result of the breakdown of carbohydrates into sugars. It enters cells through specific transporter proteins that move it from outside the cell into the cell's cytosol. All of the glycolytic enzymes are found in the cytosol.

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^+

Step 1: Hexokinase





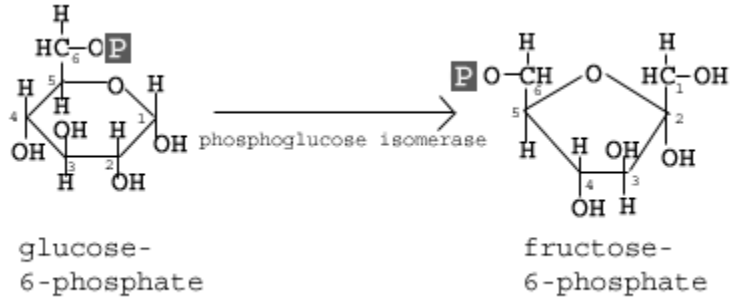
The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.

Details:

Here, the glucose ring is phosphorylated. Phosphorylation is the process of adding a phosphate group to a molecule derived from ATP. As a result, at this point in glycolysis, 1 molecule of ATP has been consumed.

The reaction occurs with the help of the enzyme hexokinase, an enzyme that catalyzes the phosphorylation of many six-membered glucose-like ring structures. Atomic magnesium (Mg) is also involved to help shield the negative charges from the phosphate groups on the ATP molecule. The result of this phosphorylation is a molecule called glucose-6-phosphate (G6P), thusly called because the 6′ carbon of the glucose acquires the phosphate group.

Step 2: Phosphoglucose Isomerase



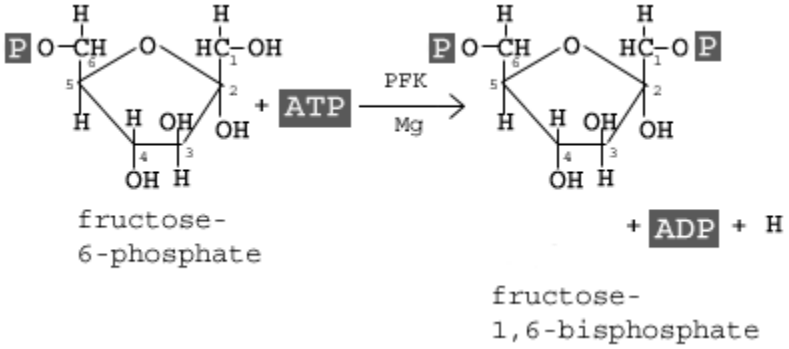
The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase (Phosphoglucose Isomerase).

Details:

The second step of glycolysis involves the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction occurs with the help of the enzyme phosphoglucose isomerase (PI). As the name of the enzyme suggests, this reaction involves an isomerization reaction.

The reaction involves the rearrangement of the carbon-oxygen bond to transform the six-membered ring into a five-membered ring. To rearrangement takes place when the six-membered ring opens and then closes in such a way that the first carbon becomes now external to the ring.

Step 3: Phosphofructokinase



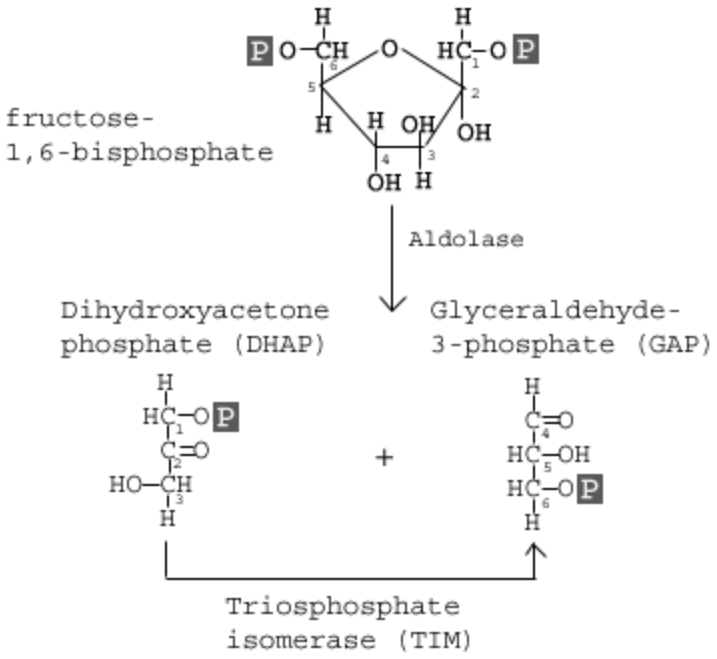
Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate.

Details:

In the third step of glycolysis, fructose-6-phosphate is converted to fructose- 1,6-*bi*sphosphate (FBP). Similar to the reaction that occurs in step 1 of glycolysis, a second molecule of ATP provides the phosphate group that is added on to the F6P molecule.

The enzyme that catalyzes this reaction is phosphofructokinase (PFK). As in step 1, a magnesium atom is involved to help shield negative charges.

Step 4: Aldolase

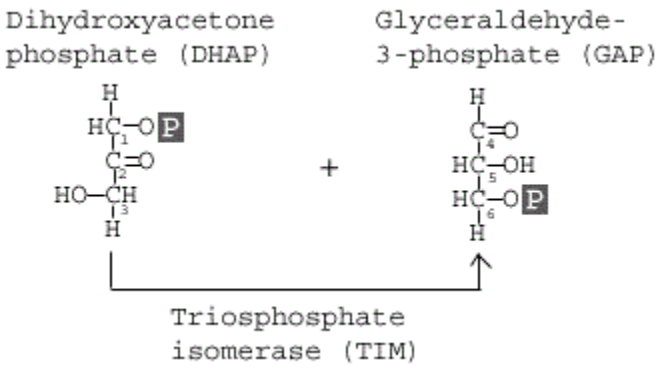


The enzyme Aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

Details:

This step utilizes the enzyme aldolase, which catalyzes the cleavage of FBP to yield two 3-carbon molecules. One of these molecules is called glyceraldehyde-3-phosphate (GAP) and the other is called dihydroxyacetone phosphate (DHAP).

Step 5: Triphosphate isomerase

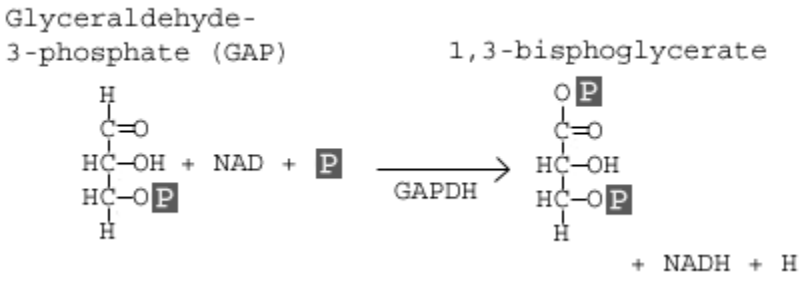


The enzyme triophosphate isomerase rapidly inter- converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed / used in next step of Glycolysis.

Details:

GAP is the only molecule that continues in the glycolytic pathway. As a result, all of the DHAP molecules produced are further acted on by the enzyme triphoshpate isomerase (TIM), which reorganizes the DHAP into GAP so it can continue in glycolysis. At this point in the glycolytic pathway, we have two 3-carbon molecules, but have not yet fully converted glucose into pyruvate.

Step 6: Glyceraldehyde-3-phosphate Dehydrogenase



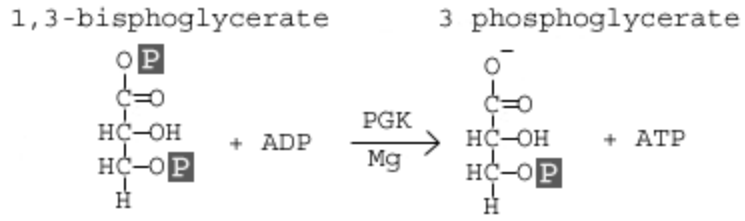
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate.

Details:

In this step, two main events take place: 1) glyceraldehyde-3-phosphate is oxidized by the coenzyme nicotinamide adenine dinucleotide (NAD); 2) the molecule is phosphorylated by the addition of a free phosphate group. The enzyme that catalyzes this reaction is glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The enzyme GAPDH contains appropriate structures and holds the molecule in a conformation such that it allows the NAD molecule to pull a hydrogen off the GAP, converting the NAD to NADH. The phosphate group then attacks the GAP molecule and releases it from the enzyme to yield 1,3 bisphoglycerate, NADH, and a hydrogen atom.

Step 7: Phosphoglycerate Kinase



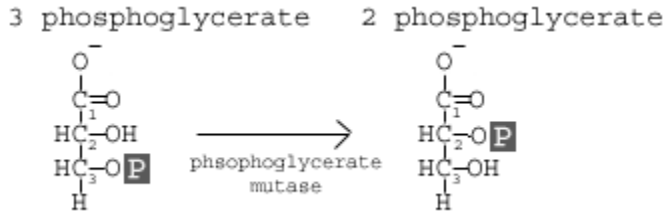
Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

Details:

In this step, 1,3 bisphoglycerate is converted to 3-phosphoglycerate by the enzyme phosphoglycerate kinase (PGK). This reaction involves the loss of a phosphate group from the starting material. The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP. Since we actually have two molecules of 1,3 bisphoglycerate (because there were two 3-carbon products from stage 1 of glycolysis), we actually synthesize two molecules of ATP at this step. With this synthesis of ATP, we have cancelled the first two molecules of ATP that we used, leaving us with a net of 0 ATP molecules up to this stage of glycolysis.

Again, we see that an atom of magnesium is involved to shield the negative charges on the phosphate groups of the ATP molecule.

Step 8: Phosphoglycerate Mutase



The enzyme phosphoglycero mutase relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

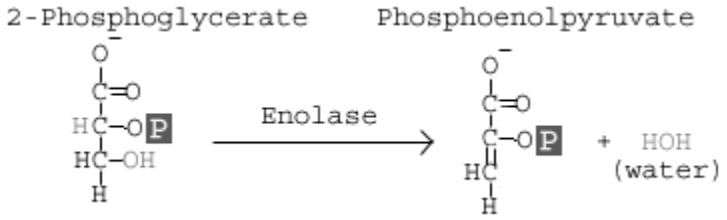
Details:

This step involves a simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate molecule, making it 2 phosphoglycerate. The molecule responsible for

catalyzing this reaction is called phosphoglycerate mutase (PGM). A *mutase* is an enzyme that catalyzes the transfer of a functional group from one position on a molecule to another.

The reaction mechanism proceeds by first adding an additional phosphate group to the 2′ position of the 3 phosphoglycerate. The enzyme then removes the phosphate from the 3′ position leaving just the 2′ phosphate, and thus yielding 2 phsophoglycerate. In this way, the enzyme is also restored to its original, phosphorylated state.

Step 9: Enolase

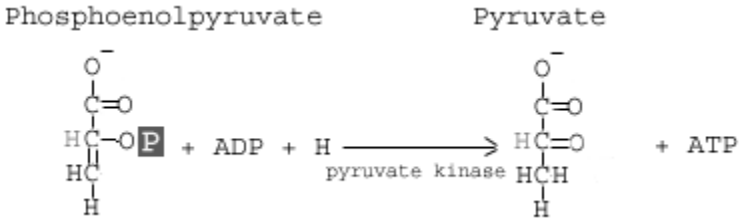


The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP).

Details:

This step involves the conversion of 2 phosphoglycerate to phosphoenolpyruvate (PEP). The reaction is catalyzed by the enzyme enolase. Enolase works by removing a water group, or *dehydrating* the 2 phosphoglycerate. The specificity of the enzyme pocket allows for the reaction to occur through a series of steps too complicated to cover here.

Step 10: Pyruvate Kinase



The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10.

Details:

The final step of glycolysis converts phosphoenolpyruvate into pyruvate with the help of the enzyme pyruvate kinase. As the enzyme's name suggests, this reaction involves the transfer of a phosphate group. The phosphate group attached to the 2′ carbon of the PEP is transferred to a molecule of ADP, yielding ATP. Again, since there are two molecules of PEP, here we actually generate 2 ATP molecules.

Q.3 B)

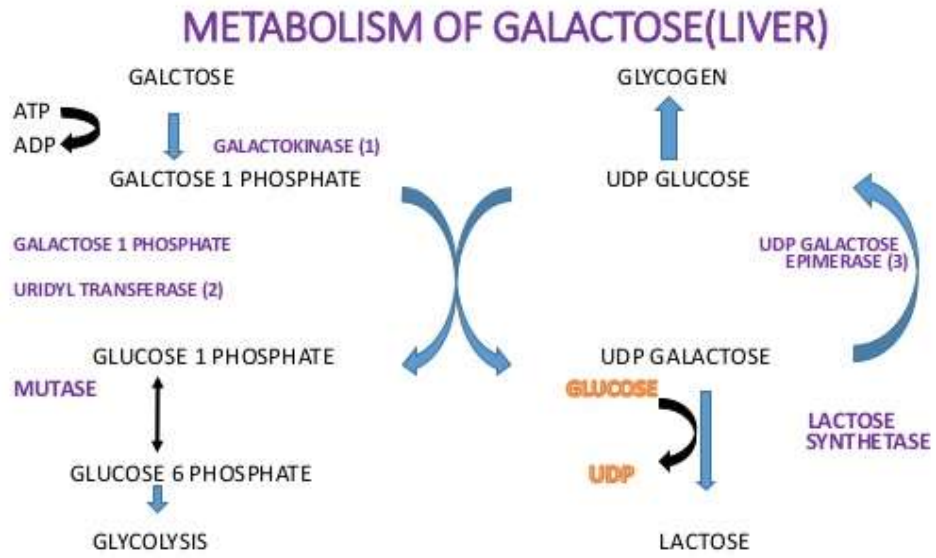
1.The pyruvate dehydrogenase complex links glycolysis to the TCA cycle (also known as the Krebs cycle or the citric acid cycle). It is a large multi-enzyme complex composed of three enzymes involving five cofactors. The pyruvate dehydrogenase complex oxidizes pyruvate to generate acetyl-coA.

The oxidation of pyruvate occurs in the mitochondria of the cell. The mitochondria is an organelle in the cell. It is considered the "powerhouse" of the cell. Pyruvate is transported there via pyruvate translocase. Pyruvate dehydrogenase is a multi-enzyme complex that uses three enzymes:

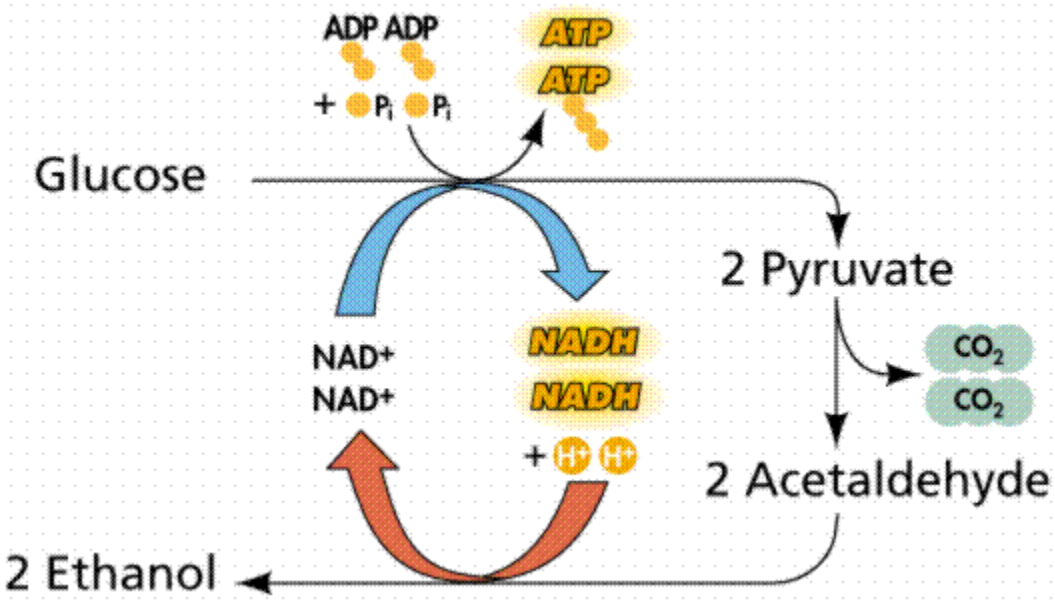
1. E_1: Pyruvate dehydrogenase which uses thiamine pyrophosphate (TPP) as its prosthetic group.
2. E_2: Dihydrolipoyl transacetylase which uses lipoamide and coenzyme A (also known as coASH) as its prosthetic groups.
3. E_3: Dihydrolipoyl dehydrogenase which uses flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD^+) as its cofactors.

2pyruvate+2NAD++2coA→2acetyl coA+2NADH+2CO2(1)(1)2pyruvate+2NAD++2coA→2acetyl coA+2NADH+2CO2

Ans.2. **Galactose Metabolism**



3. Alcoholic Fermentation:



4. Metabolic Regulation :

Regulation occurs at the three reactions far from equilibrium
Remember that at equilibrium the rates of forward and reverse reactions are equal. Therefore, the conversion of, for example, 3- phosphoglycerate to glyceraldehyde-3-phosphate occurs rapidly. In contrast, the reactions far from equilibrium, such as the conversion of phosphoenol pyruvate to pyruvate, have rates that are greater in the forward than in the reverse direction. Imagine a

series of pools in a fountain. If two pools are at the same level, there is no point in putting a dam between them to control the flow of water. On the other hand, the rate of water flow can be controlled effectively at any point where one pool spills into a lower one. Think of the compounds in the free energy diagram as pools—where does a pool spill into a lower one, offering the possbility of control? At three enzyme-catalyzed reactions:

1.Glucose-6-phosphate formation. The entry point of glucose is the formation of glucose-6-phosphate. Hexokinase is feedback-inhibited by its product, so the phosphorylation of glucose is inhibited if there is a buildup of glucose-6- phosphate. In mammalian cells, the breakdown of glycogen is regulated by covalent modification of glycogen phosphorylase. This regulation reduces the rate of formation of glucose-6-phosphate.

2.Fructose-6-phosphate ➪ fructose-1,6-bisphosphate. Glucose-6-phosphate has other metabolic fates than simply leading to pyruvate. For example, it can be used to synthesize ribose for DNA and RNA nucleotides. The most important regulatory step of glycolysis is the phosphofructokinase reaction. Phosphofructokinase is regulated by the energy charge of the cell—that is, the fraction of the adenosine nucleotides of the cell that contain high-energy bonds. Energy charge is given by the formula:

$$[ATP] + \tfrac{1}{2}[ADP]/([ATP] + [ADP] + [AMP])$$

The energy charge of a cell can vary from about 0.95 to 0.7. *ATP inhibits* the phosphofructokinase reaction by raising the K_m for fructose-6-phosphate. AMP activates the reaction. Thus, when energy is required, glycolysis is activated. When energy is plentiful, the reaction is slowed down.

Phosphofructokinase is also activated by fructose-2,6- bisphosphate, which is formed from fructose-1-phosphate by a second, separate phosphofructokinase enzyme—phosphofructokinase II (as shown in Figure). The activity of PFK II is itself regulated by hormone action. Fructose-2,6-bisphosphate allosterically activates PFK I by decreasing the K_m for fructose-6-phosphate.

Finally, phosphofructokinase is *inhibited by citrate*. Citrate is the TCA cycle intermediate where 2-carbon units enter the cycle. A large number of compounds—for example, fatty acids and amino acids—can be metabolized to TCA cycle intermediates. High concentrations of citrate indicate a plentiful supply of intermediates for energy production; therefore, high activity of the glycolytic pathway is not required.

3.Phosphoenol pyruvate → pyruvate. The third big step in the free-energy diagram is the pyruvate-kinase reaction, where ATP is formed from phosphoenol pyruvate. *ATP inhibits* pyruvate kinase, similar to the inhibition of PFK. Pyruvate kinase is also *inhibited by acetyl-Coenzyme A*, the product of pyruvate metabolism that enters the TCA cycle. Fatty acids also allosterically inhibit pyruvate kinase, serving as an indicator that alternative energy sources are available for the cell.

Pyruvate kinase is also *activated by fructose-1,6-bisphosphate*. Why fructose-1,6-bisphosphate? It is an example of feed-forwardactivation. This glycolytic intermediate is controlled by its own enzyme system. If glycolysis is activated, then the activity of pyruvate kinase must also be increased in order to allow overall carbon flow through the pathway. Feed-forward activation ensures that the enzymes act in concert to the overall goal of energy production.

Q.4. A) Answer any one of the following: (10)

1. Explain in detail the series of reaction involved in β oxidation of Myristic acid.

Oxidation of fatty acids occurs in three stages:

- **β-oxidation** of fatty acids resulting in cleavage of two-carbon units (**α and β carbons**) from the carboxyl end of **fatty acyl-CoA** with the formation of **acetyl CoA**. This reaction keeps occuring till the entire fatty acyl chain is broken down to acetyl CoA molecules. For eg. Myristoyl CoA (14 carbon chain) on β-oxidation will give seven acetyl CoA molecules.
- The **acetyl groups** produced from β-oxidation of the fatty acid participate in the **Kreb's cycle** resulting in the formation of NADH and $FADH_2$.
- The reduced coenzymes (**NADH and $FADH_2$**) are oxidized by giving up the protons and electrons to oxygen present in the mitochondria to synthesize ATP by **oxidative phosphorylation** in the Electron Transport System.

Once the fatty acids have been transported to the mitochondrial matrix via carnitine pathway, **β-oxidation** of **fatty acyl-CoA (n carbons)** occurs within the mitochondria in four steps as discussed below:

- **First step**– **Fatty acyl-CoA** is acted upon by an enzyme ***acyl-CoA dehydrogenase*** which is FAD dependent. **Fatty acyl-CoA**undergoes **dehydrogenation** and forms a **trans-double bond** at the **α and β carbons** to form **trans-Δ^2-enoyl-CoA**. ***Acyl-CoA dehydrogenase*** are present as three isoenzymes each specific for a particular carbon chain length (short, intermediate and long). The electrons which were removed from the fatty acyl-CoA chain are transferred to **FAD which gets reduced to $FADH_2$**. This $FADH_2$ immediately via the Electron Transport System gets converted to ATP molecules.

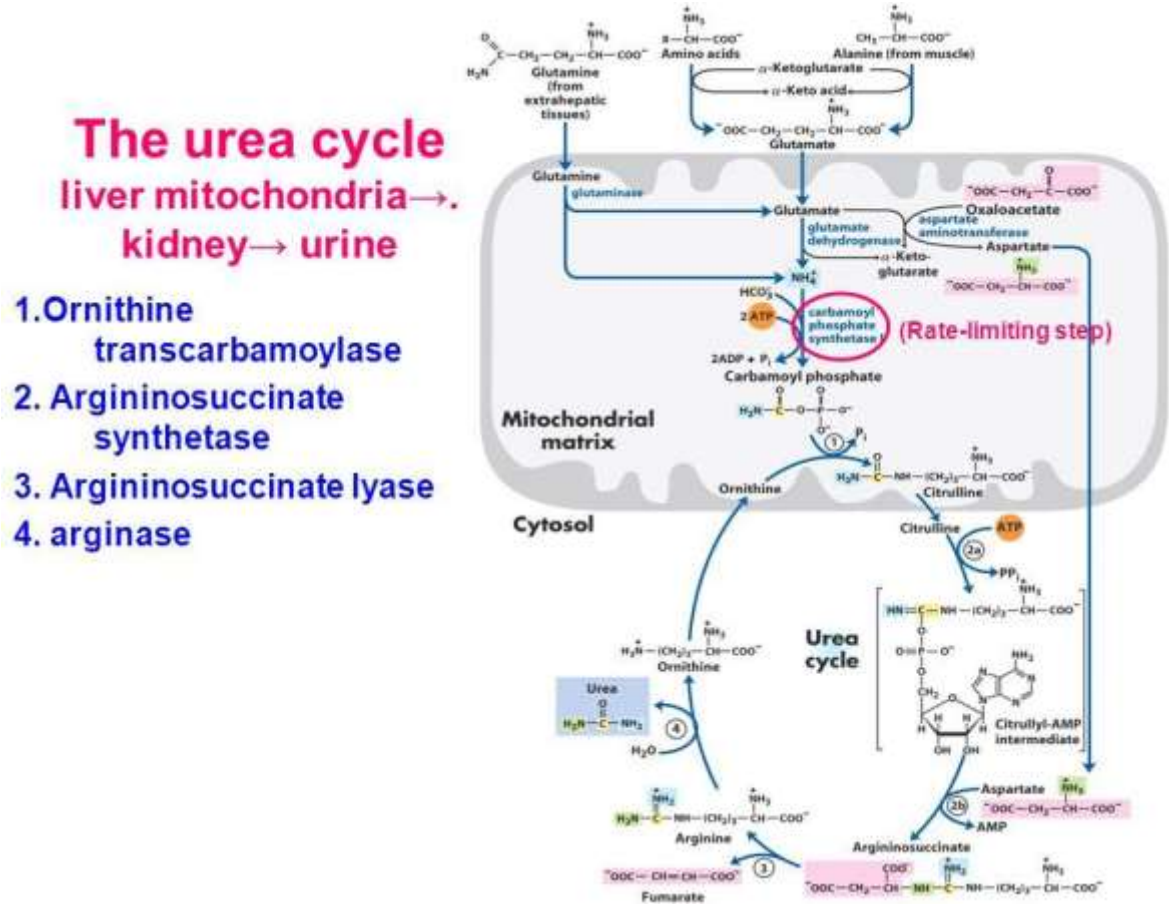
- **<u>Second step</u>**– ***Enoyl-CoA hydratase*** catalyzes this reaction where **water** is added. **Hydration** occurs at the double bond resulting in the formation of **β-hydroxyacyl-CoA**.
- **<u>Third step</u>**– **β-hydroxyacyl-CoA** undergoes **dehydrogenation** to form **β-ketoacyl-CoA** in the presence of ***β-hydroxyacyl-CoA dehydrogenase***. The electrons available as a result of dehydrogenation are accepted by **NAD^+ to form NADH + H^+** which immediately exchanges these electrons with oxygen in the Electron Transport System to form ATP molecules.
- **<u>Fourth step</u>**– This reaction is called as **thiolysis** as ***acyl-CoA acetyltransferase***(also known as ***thiolase***) in the presence of **CoA-SH** causes the **cleavage** of **β-ketoacyl-CoA** to form **acetyl CoA** and the **thioester of the original fatty acid with two carbons less**. This cleavage occurs as the β carbon ketone group is a good target for nucleophilic attack by the thiol (-SH) group of the coenzyme A.

The **new fatty acyl-CoA (n-2 carbons)** formed again participates in the β-oxidation cycle to form a new fatty acyl-CoA with two carbons less (n-4 carbons) and a new molecule of acetyl CoA. This process continues till the entire fatty acid is converted into acetyl CoA molecules.

Acetyl CoA formed from the above steps now enters the **<u>Kreb's cycle</u>** to get oxidized to CO_2 and H_2O.

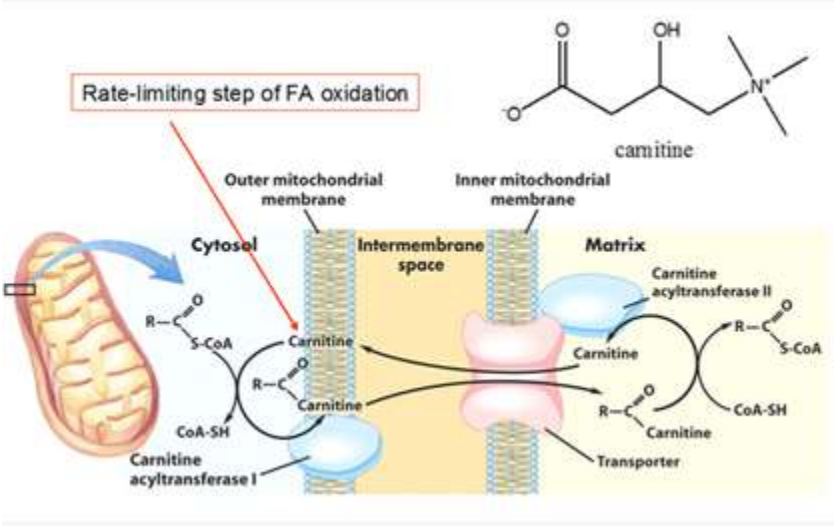
-

2. Describe the various steps required to form Urea.



Q. 4. B) Answer any two of the following: (10)

i. Describe the Carnitine shuttle.



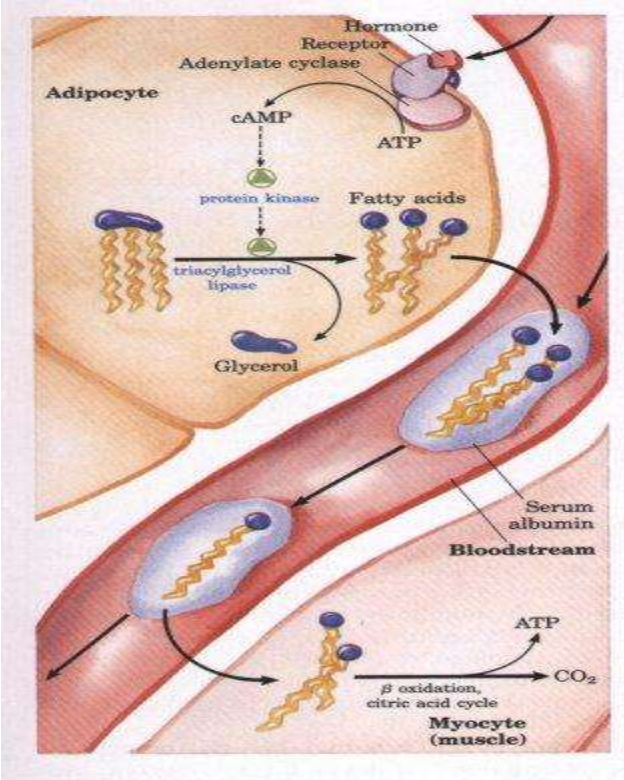
Fatty acyl-CoA esters formed in the outer mitochondrial membrane do not cross the inner mitochondrial membrane intact. Instead, the fatty acyl group is transiently attached to the

hydroxyl group of **carnitine** and the fatty acyl-carnitine is carried across the inner mitochondrial membrane by a specific transporter (Fig. 16-6). In this second enzymatic reaction required for fatty acid movement into mitochondria, **carnitine acyltransferase I**, present on the outer face of the inner membrane, catalyzes transesterification of the fatty acyl group from coenzyme A to carnitine. The fatty acyl-carnitine ester crosses the inner mitochondrial membrane into the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter**.

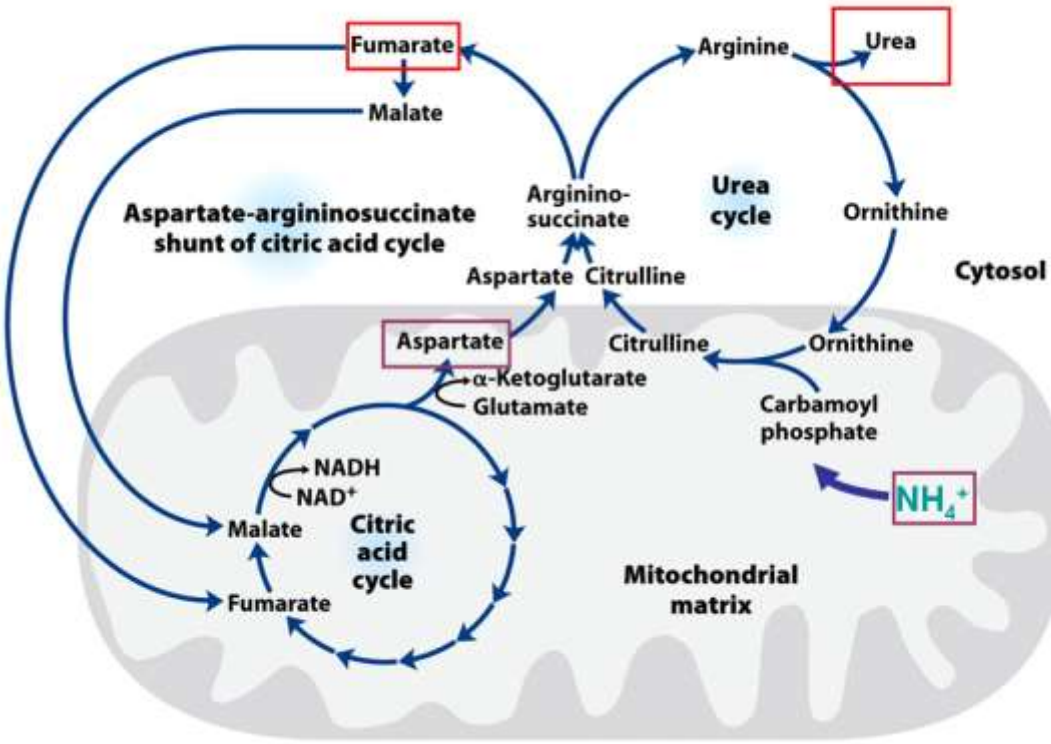
In the third and final step of the entry process, the fatty acyl group is enzymatically transferred from carnitine to intramitochondrial coenzyme A by **carnitine acyltransferase II**. This isozyme is located on the inner face of the inner mitochondrial membrane, where it regenerates fatty acyl-CoA and releases it, along with free carnitine, into the matrix. Carnitine reenters the space between the inner and outer mitochondrial membranes via the acyl-carnitine/carnitine transporter.

ii. How are triacylglcerols mobilized in adipose tissue?

When hormones signal the need for metabolic energy, triacylglycerols stored in adipose tissue are mobilized (brought out of storage) and transported to those tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be'oxidized for energy production. The hormones epinephrine and glucagon, secreted in response to low blood glucose levels, activate adenylate cyclase in the adipocyte plasma membrane (Fig. 16-3), raising the intracellular concentration of cAMP (see Fig. 14-18). A cAMP-dependent protein kinase, in turn, phosphorylates and thereby activates **hormone-sensitive triacylglycerol lipase**, which catalyzes hydrolysis of the ester linkages of triacylglycerols. The fatty acids thus released diffuse from the adipocyte into the blood, where they bind to the blood protein **serum albumin**. This protein (Mr 62,000), which constitutes about half of the total serum protein, binds as many as 10 fatty acids per protein monomer by noncovalent interactions. Bound to this soluble protein, the otherwise insoluble fatty acids are carried to tissues such as skeletal muscle, heart, and renal cortex. Here, fatty acids dissociate from albumin and diffuse into the cytosol of the cells in which they will serve as fuel.



iii. Explain Krebs bicycle.



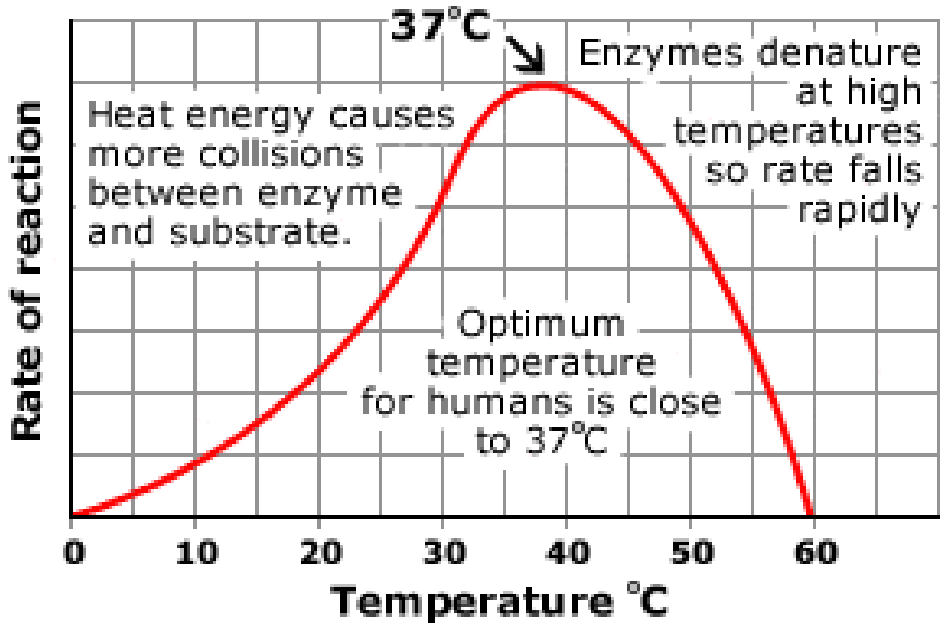
iv. Describe the process of transport of ammonia in the form of glutamine.

Q. 5 Write short notes on any four of the following: **20**

a. Temperature as a factor affecting enzyme activity:

Temperature: At **low temperatures**, enzymes have **low activity**. As the temperature rises the **rate of reaction increases**, usually **2-fold for every 10 degree Celsius rise.**

The activity peaks at a specific temperature unique to the enzyme. This is known as the **optimum temperature** - the temperature at which an enzyme is maximally active. Beyond the optimum temperature the activity of the enzyme decreases. At extreme temperatures, the enzymes are **denatured** and activity ceases. Any suitable example can be given.



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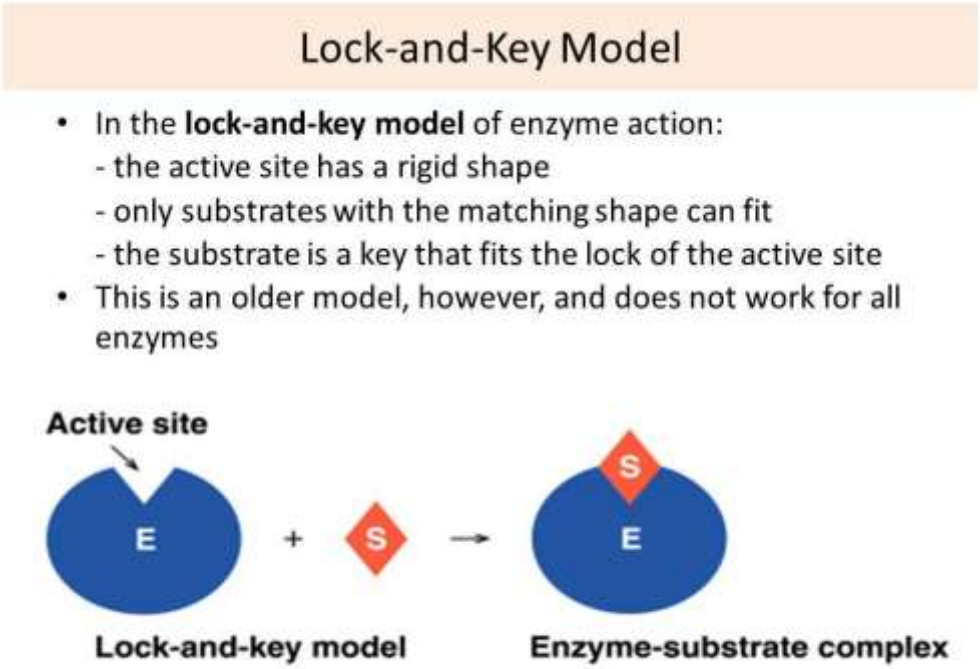
Q 5 b. Lock and Key Model of enzyme action:



c.Complex I - NADH-coenzyme Q oxidoreductase

The reduced coenzyme NADH binds to this complex, and functions to reduce coenzyme Q10. This reaction donates electrons, which are then transferred through this complex using FMN (Flavin mononucleotide) and a series of Fe-S (Iron-sulpur) clusters. The transport of these electrons brings about the transfer of protons across the membrane into the intermembrane space.

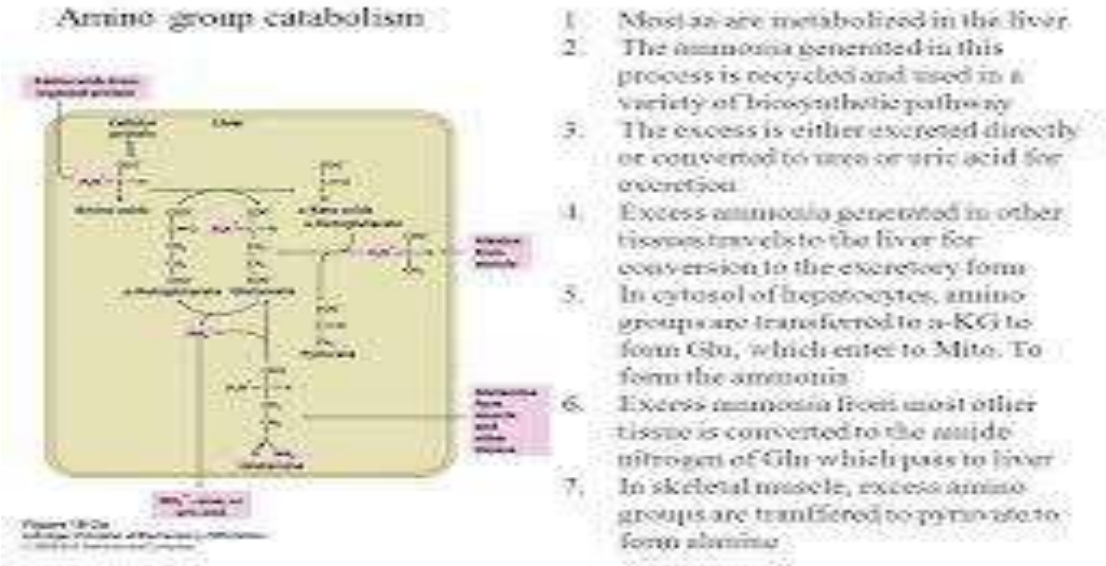
d. **Substrate-Level Phosphorylation**

Inside the mitochondria is where most of your ATP are created, but some ATP can be made in the cytoplasm through a process called **substrate-level phosphorylation**. This is a process of forming ATP by the physical addition of a phosphate group to ADP. Substrate-level phosphorylation is a big name, but it's actually a straightforward process. With this type of phosphorylation you have an adenosine diphosphate (ADP), which is a unit of adenosine attached to two (*di*) phosphate groups. In order to turn it into an adenosine triphosphate, a phosphate group is taken from an intermediate compound, referred to as a substrate, and given to the ADP.

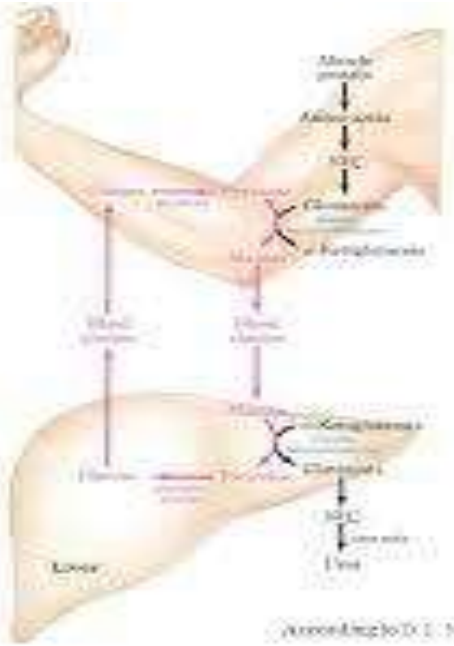
I mentioned that substrate-level phosphorylation takes place outside of the mitochondria, but it also takes place inside. When it takes place outside in the cytoplasm, it is occurring during **glycolysis**, which is the breakdown of glucose to produce pyruvate and energy.

Glycolysis does not require oxygen, but oxygen is an important part of ATP production inside the mitochondria. Substrate-level phosphorylation inside the mitochondria occurs during the **Krebs cycle**, also referred to as the citric acid cycle. This cycle is a sequence of chemical reactions in which molecules are broken down in the presence of oxygen to generate energy. The Krebs cycle takes place inside the folded inner membrane of the mitochondria, in an area known as the matrix.

e. Role played by Glutamate, Glutamic acid and Alanine as nitrogen carriers.



f. Glucose – Alanine cycle.



Glucose-alanine cycle

Alanine plays a special role in transporting amino groups to liver.

Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.
The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.