AS- 2240
Model Answer

Course : M.Sc. Biotechnology
Semester : I Semester
Subject : Biochemistry (Metabolism and Regulation)
Subject Code : LBTM-104

A. Objective type questions

1. (d) Oxidative phosphorylation
2. (b) $6CO_2+6H_2O \rightarrow C_6H_{12}O_6+6O_2$
3. (d) insulin
4. (b)Two
5. (d) Fumarate
6. (a) α-Ketoglutarate
7. (c) Both (a) and (b)
8. (b) Two-SH groups
9. (a) Adenosine kinase
10. (a) PRPP

B. Attempt any four of the followings

Ans. B. 2.

Substrate-level phosphorylation

A specific type of metabolic reaction involving phosphate-containing intermediates. Substrate-level phosphorylation entails a direct coupling of an energy-liberating biochemical reaction with the energy-conserving formation of a "high energy" compound, typically adenosine triphosphate, or ATP. An example of substrate-level phosphorylation occurs in glycolysis when phosphoenolpyruvate (PEP) reacts with ADP to form ATP and pyruvate, a reaction catalyzed by the enzyme pyruvate kinase.

Phosphoryl transfer potential, like any form of potential energy, can be measured relative to a reference. Here, the hydrolysis reaction with water - that is, phosphoryl group transfer to water - serves as a reference. The hydrolysis reaction is effectively always a thermodynamically favorable process, with biochemical free energy change negative in sign ($\Delta G^{\circ\prime} < 0$). The greater the phosphoryl transfer potential of a given compound bearing a phosphoryl group, the more energetically favorable is its hydrolysis reaction. In the case of PEP, hydrolysis would release a considerable amount of energy. In the metabolic context of glycolysis, instead of undergoing hydrolysis, PEP transfers its phosphoryl group to ADP, thereby conserving much of the energy of PEP hydrolysis in the form of a phosphoric anhydride bond in ATP.

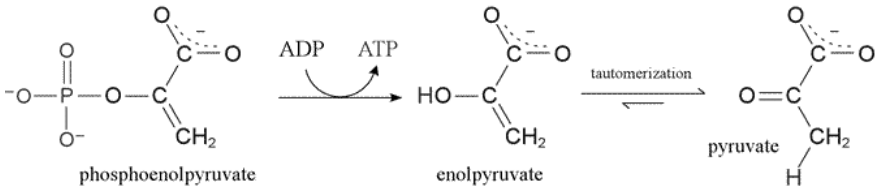
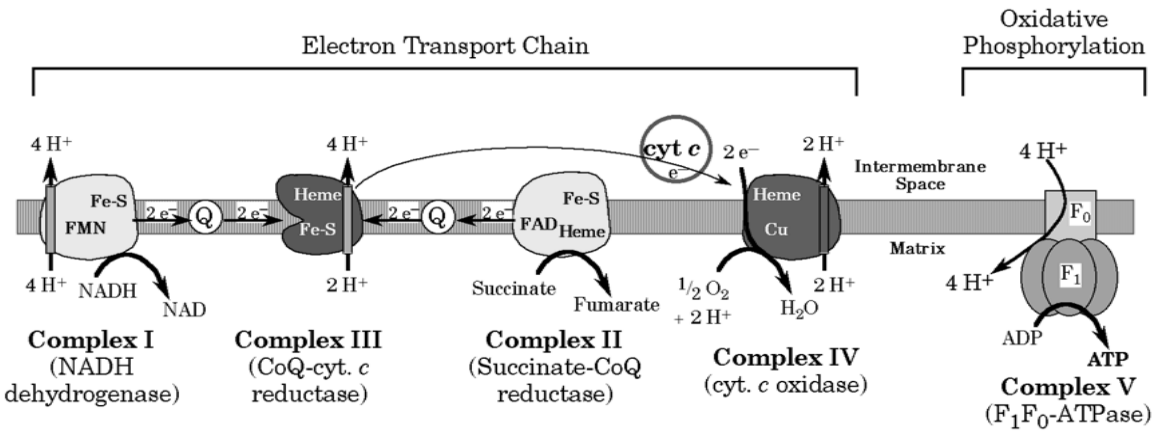
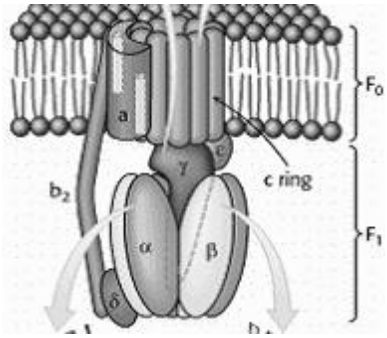


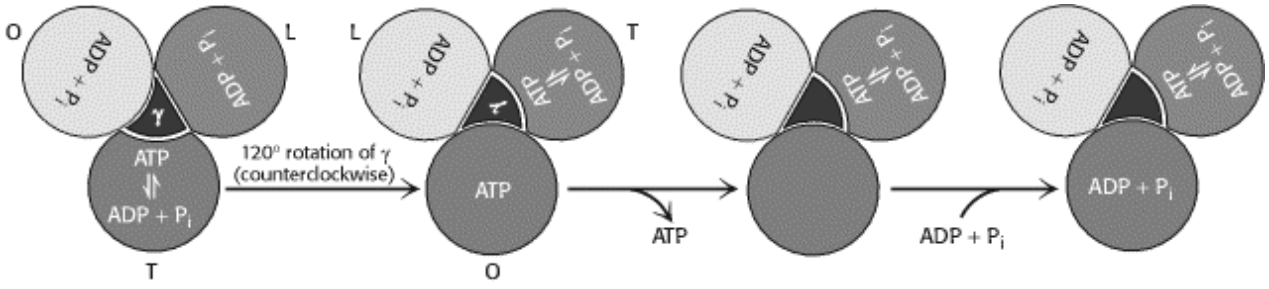
Diagram of substrate-level phosphorylation reaction involving phosphoenolpyruvate (PEP)\

Oxidative phosphorylation is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). Although the many forms of life on earth use a range of different nutrients, almost all carry out oxidative phosphorylation to produce ATP, the molecule that supplies energy to metabolism. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.



During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within mitochondria, whereas, in prokaryotes, these proteins are located in the cells' inner membranes. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.





The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called chemiosmosis. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and

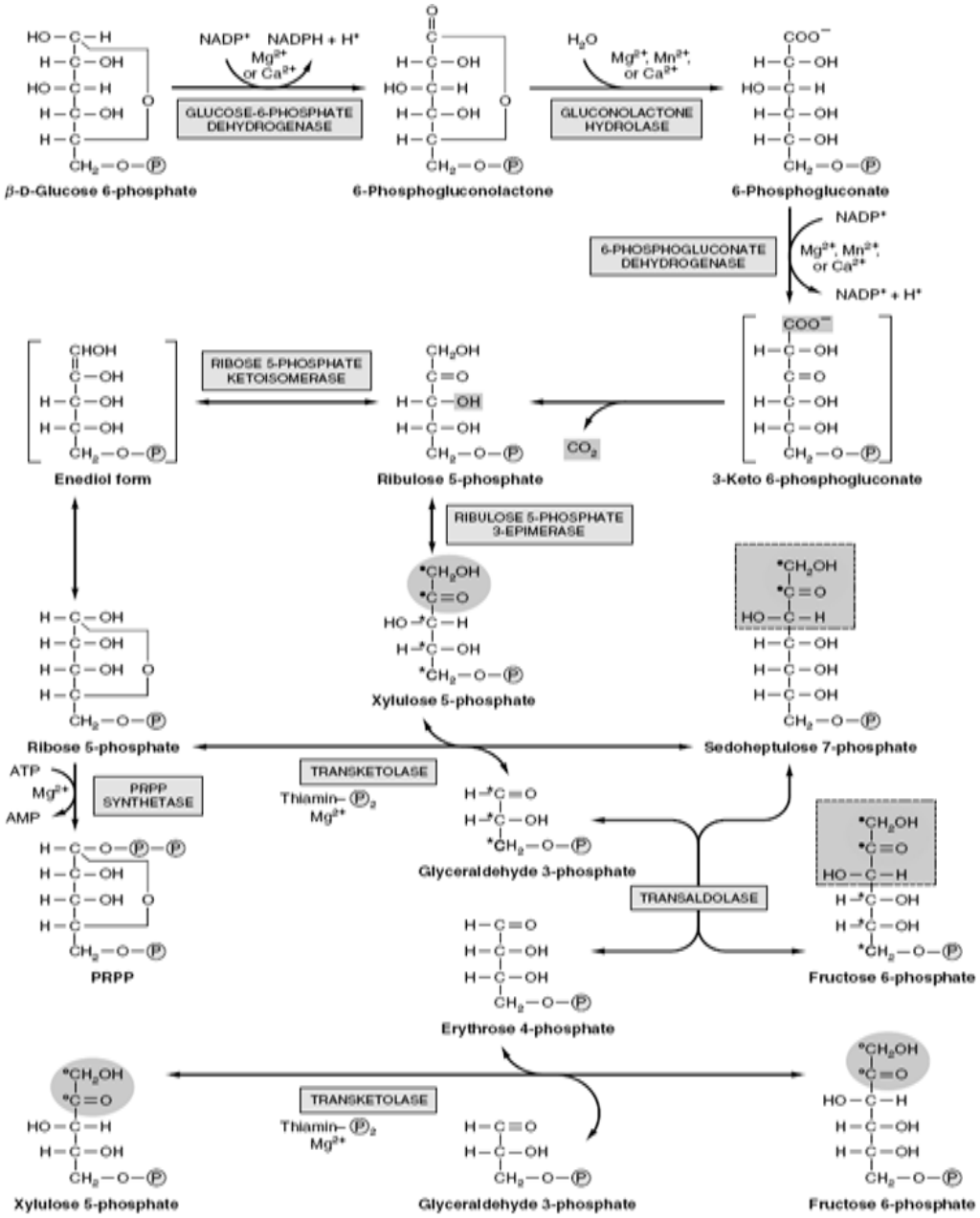
contributing to disease and, possibly, aging (senescence). The enzymes carrying out this metabolic pathway are also the target of many drugs and poisons that inhibit their activities.

Ans. B.3.

The pentose phosphate pathway is an alternative route for the metabolism of glucose. It does not generate ATP but has two major functions:

(1) The formation of **NADPH** for synthesis of fatty acids and steroids and

(2) the synthesis of **ribose** for nucleotide and nucleic acid formation. Glucose, fructose, and galactose are the main hexoses absorbed from the gastrointestinal tract, derived principally from dietary starch, sucrose, and lactose, respectively. Fructose and galactose are converted to glucose, mainly in the liver.



Ans. B. 4.

Many bacteria and plants are able to subsist on acetate or other compounds that yield acetyl CoA. They make use of a metabolic pathway absent in most other organisms that converts two-carbon acetyl units into four-carbon units (succinate) for energy production and biosyntheses. This reaction sequence, called the *glyoxylate cycle*, bypasses the two decarboxylation steps of the citric acid cycle. Another key ifference is that two molecules of acetyl CoA enter per turn of the glyoxylate cycle, compared with one in the citric acid cycle.

The glyoxylate cycle, like the citric acid cycle, begins with the condensation of acetyl CoA and oxaloacetate to form citrate, which is then isomerized to isocitrate. Instead of being decarboxylated, isocitrate is cleaved by *isocitrate lyase* into succinate and glyoxylate. The subsequent steps regenerate oxaloacetate from glyoxylate. Acetyl CoA condenses with glyoxylate to form malate in a reaction catalyzed by *malate synthase*, which resembles citrate synthase. Finally, malate is oxidized to oxaloacetate, as in the citric acid cycle. The sum of these reactions is:

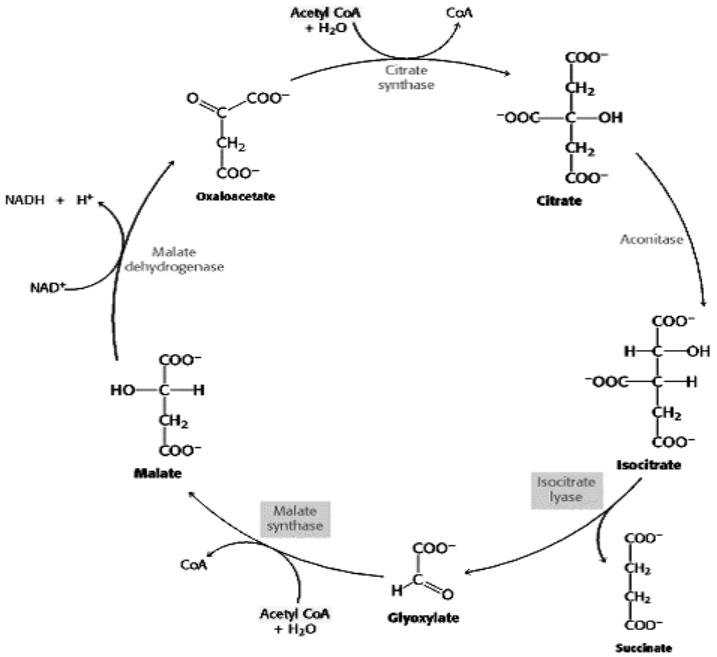
$$2\,\text{Acetyl CoA} + \text{NAD}^+ + 2\,\text{H}_2\text{O} \longrightarrow \text{succinate} + 2\,\text{CoA} + \text{NADH} + 2\,\text{H}^+$$

In plants, these reactions take place in organelles called *glyoxysomes.* Succinate, released midcycle, can be converted into carbohydrates by a combination of the citric acid cycle and gluconeogenesis. Thus, organisms with the glyoxylate cycle gain a metabolic versatility.

Bacteria and plants can synthesize acetyl CoA from acetate and CoA by an ATP-driven reaction that is catalyzed by *acetyl CoA synthetase.*

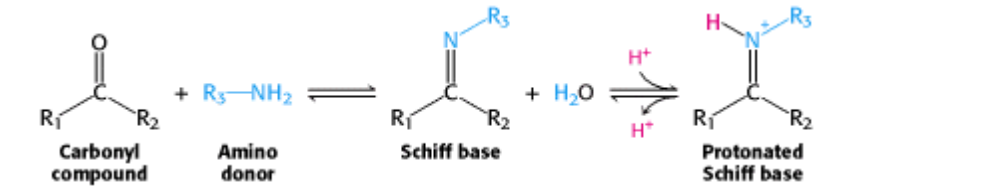
$$\text{Acetate} + \text{CoA} + \text{ATP} \longrightarrow \text{acetyl CoA} + \text{AMP} + \text{PP}_i$$

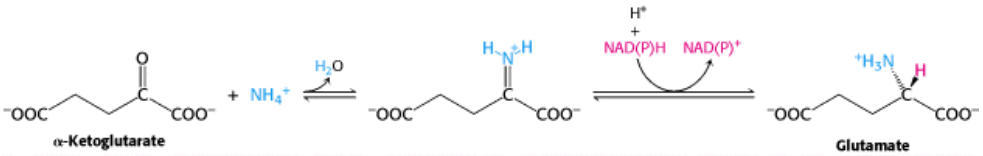
Pyrophosphate is then hydrolyzed to orthophosphate, and so the equivalents of two compounds having high phosphoryl transfer potential are consumed in the activation of acetate. We will return to this type of activation reaction in fatty acid degradation , where it is used to form fatty acyl CoA, and in protein synthesis, where it is used to link amino acids to transfer RNAs

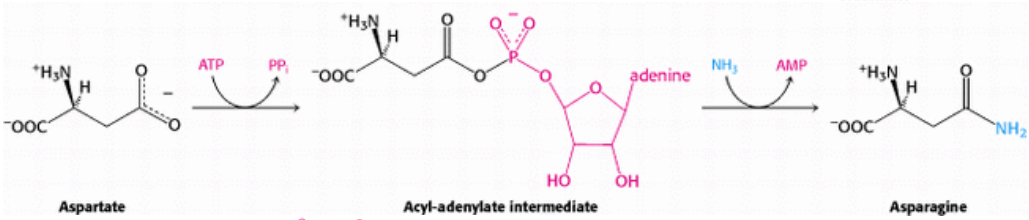


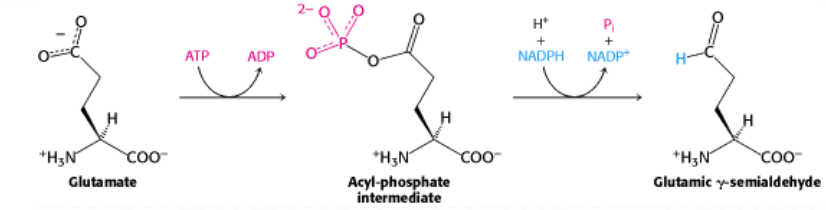
Ans. B. 5.

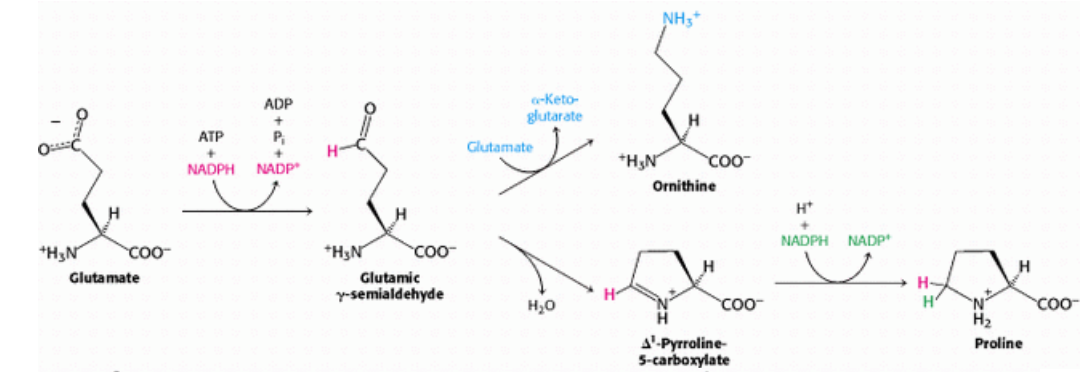
$$NH_4^+ + \alpha\text{-ketoglutarate} + NADPH + H^+ \rightleftharpoons glutamate + NADP^+ + H_2O$$

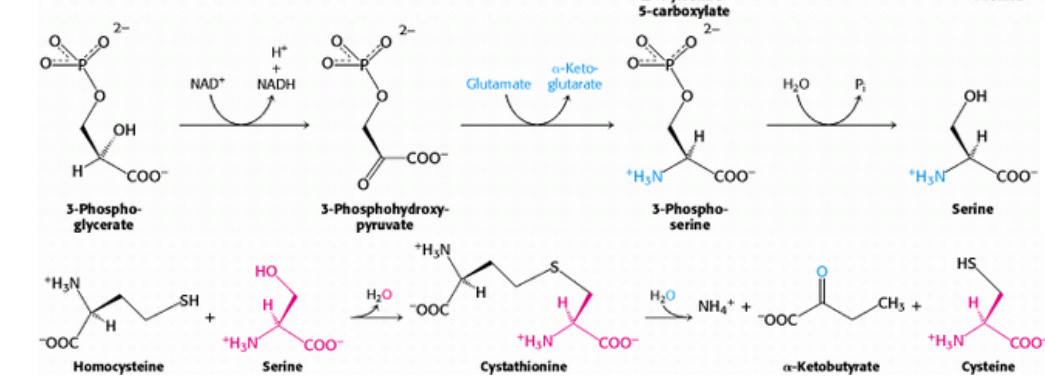


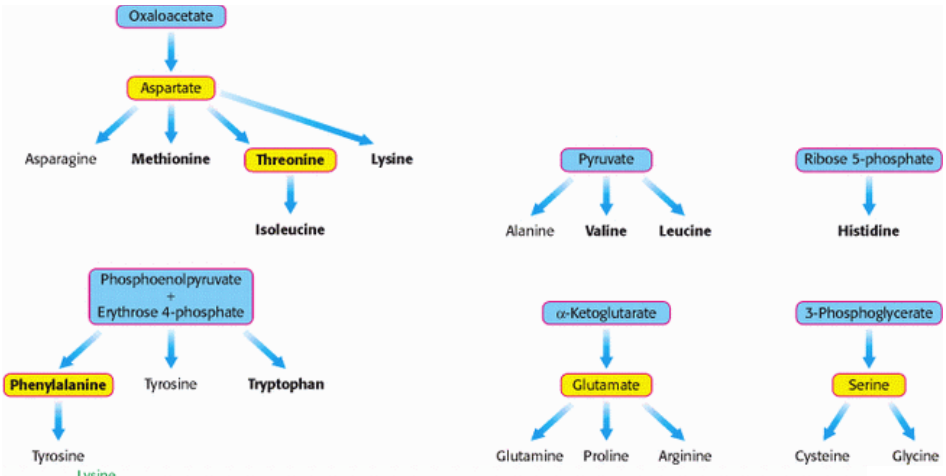


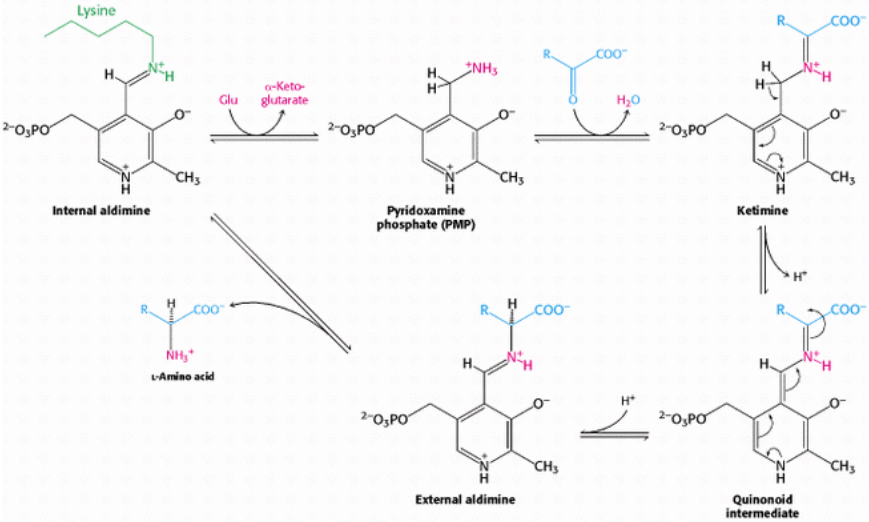


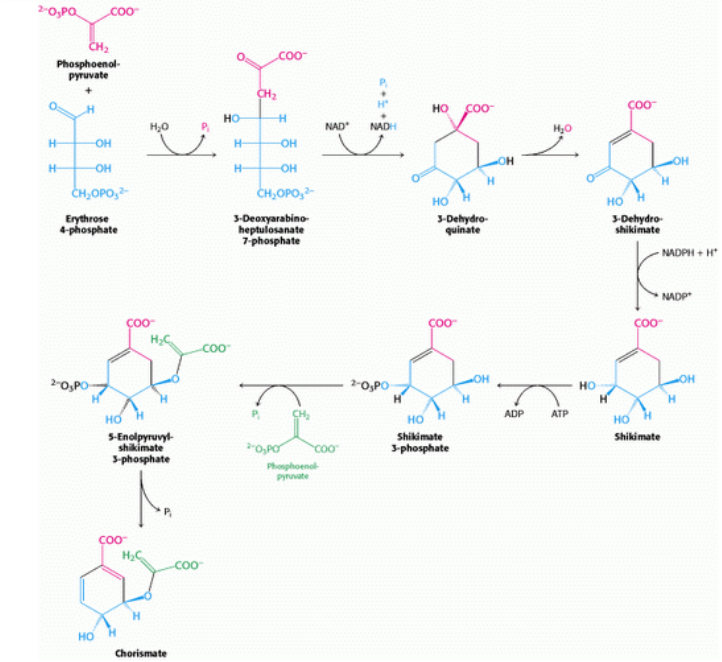


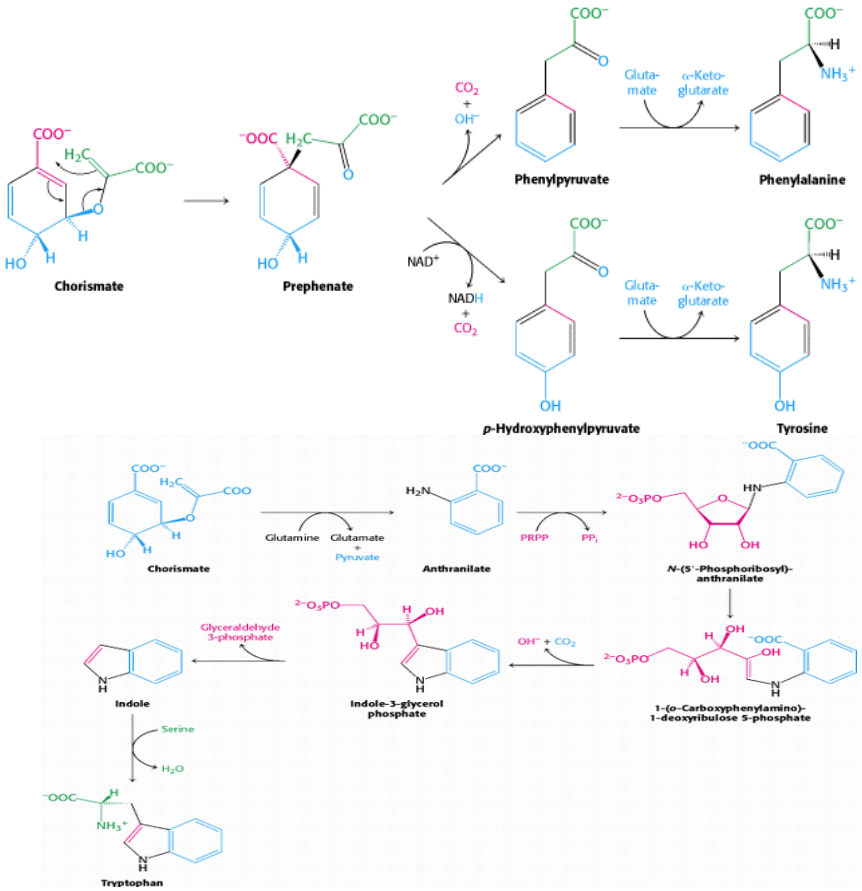





Oxaloacetate
Aspartate
Asparagine
Methionine
Threonine
Lysine
Isoleucine
Pyruvate
Alanine
Valine
Leucine
Ribose 5-phosphate
Histidine
Phosphoenolpyruvate + Erythrose 4-phosphate
Phenylalanine
Tyrosine
Tryptophan
Tyrosine
α-Ketoglutarate
Glutamate
Glutamine
Proline
Arginine
3-Phosphoglycerate
Serine
Cysteine
Glycine

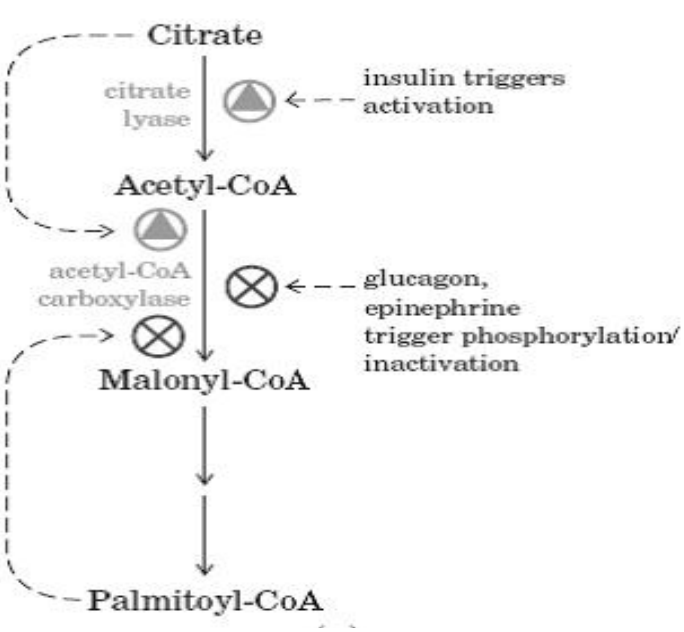

Lysine
Glu
α-Keto-glutarate
Internal aldimine
Pyridoxamine phosphate (PMP)
Ketimine
L-Amino acid
External aldimine
Quinonoid intermediate

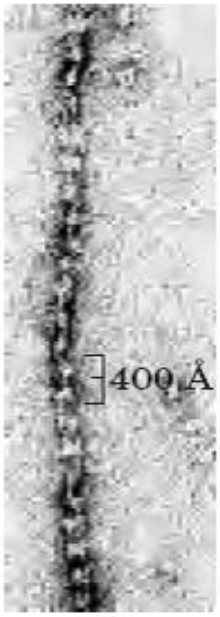

Phosphoenol-pyruvate
Erythrose 4-phosphate
3-Deoxyarabino-heptulosanate 7-phosphate
3-Dehydro-quinate
3-Dehydro-shikimate
NADPH + H+
NADP+
Shikimate
ADP
ATP
Shikimate 3-phosphate
5-Enolpyruvyl-shikimate 3-phosphate
Chorismate



Ans. B. 6.

When a cell or organism has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipids such as triacylglycerols. The reaction catalyzed by acetyl-CoAcarboxylase is the rate-limiting step in the biosynthesis of fatty acids, and this enzyme is an important site of regulation. In vertebrates, palmitoyl-CoA, the principal product of fatty acid synthesis, is a feedback inhibitor of the enzyme; citrate is an allosteric activator, increasing *V*max. Citrate plays a central role in diverting cellular metabolism from the consumption (oxidation) of metabolic fuel to the storage of fuel as fatty acids. When the concentrations of mitochondrial acetyl-CoA and ATP increase, citrate is transported out of mitochondria; it then becomes both the precursor of cytosolic acetyl-CoA and an allosteric signal for the activationof acetyl-CoA carboxylase. At the same time, citrate inhibits the activity of phosphofructokinase-1, reducing the flow of carbon through glycolysis. Acetyl-CoA carboxylase is also regulated by covalent modification. Phosphorylation, triggered by the hormones glucagon and epinephrine, inactivates the enzyme and reduces its sensitivity to activation by citrate, thereby slowing fatty acid synthesis. In its active (dephosphorylated) form, acetyl-CoA carboxylase polymerizes into long filaments; phosphorylation is accompanied by dissociation into monomeric subunits and loss of activity.



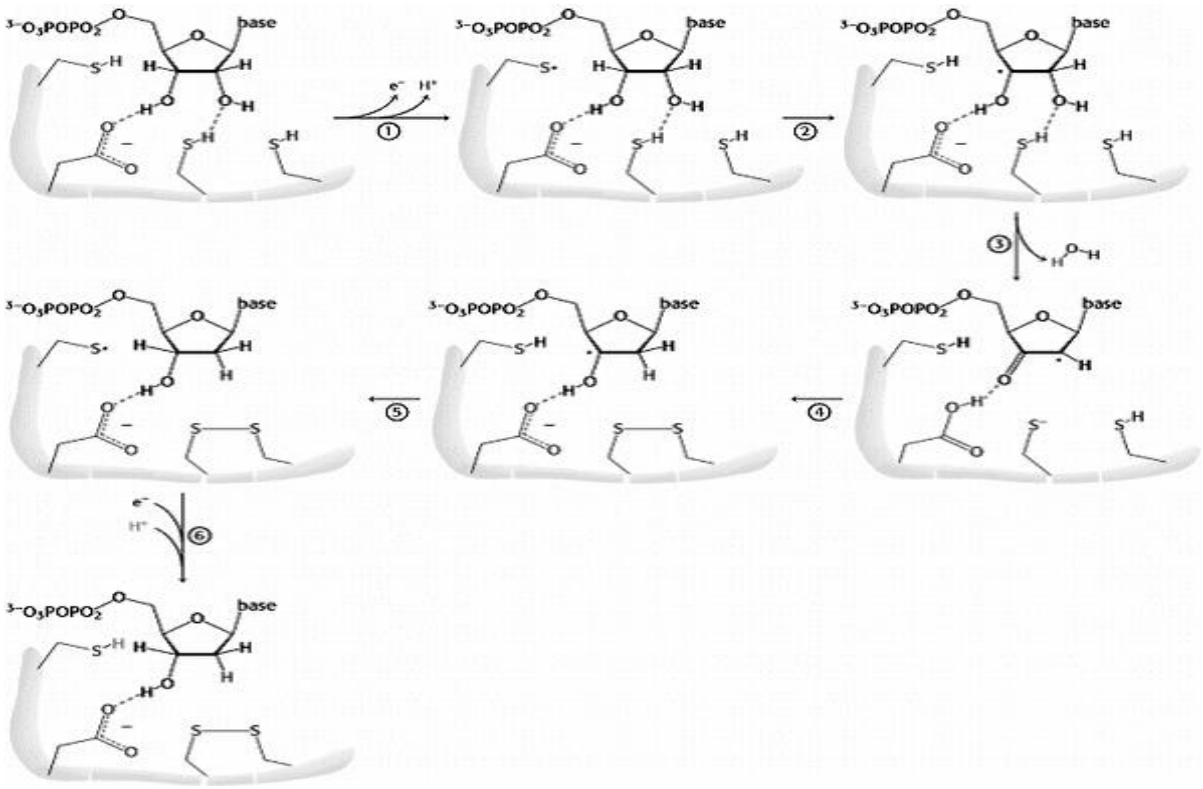


Acetyl Co-A synthase

Acetyl-CoA carboxylase is also regulated by covalent modification. Phosphorylation, triggered by the hormones glucagon and epinephrine, inactivates the enzyme and reduces its sensitivity to activation by citrate, thereby slowing fatty acid synthesis. In its active (dephosphorylated) form, acetyl-CoA carboxylase polymerizes into long filaments (Fig. 21–11b); phosphorylation is accompanied by dissociation into monomeric subunits and loss of activity.

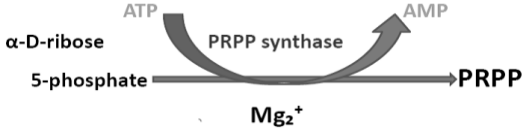
Ans. B. 7.

1. The reaction begins with the transfer of an electron from a cysteine residue on R1 to the tyrosyl radical on R2. The loss of an electron generates a highly reactive *cysteine thiyl radical* within the active site of R1.
2. This radical then abstracts a hydrogen atom from C-3 of the ribose unit, generating a radical at that carbon atom.
3. The radical at C-3 promotes the release of the hydroxide ion on the carbon-2 atom. Protonated by a second cysteine residue, the departing hydroxide ion leaves as a water molecule.
4. A hydride ion (a proton on two electrons) is then transferred from a third cysteine residue to complete the reduction of the C-2 position, form a disulfide bond, and reform a C-3 radical.
5. This C-3 radical recaptures the same hydrogen atom originally abstracted by the first cysteine residue, and the deoxyribonucleotide is free to leave the enzyme.
6. The disulfide bond generated in the enzyme's active site is then reduced by specific disulfide-containing proteins, such as thioredoxin, to regenerate the active enzyme.

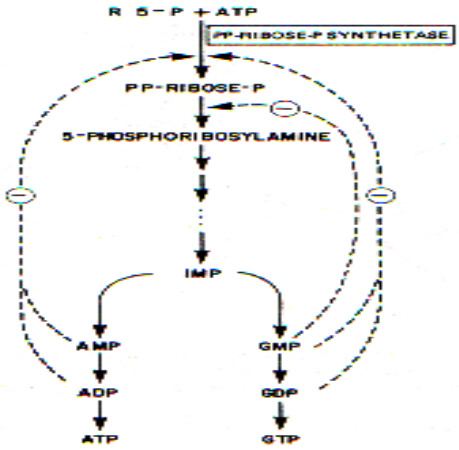


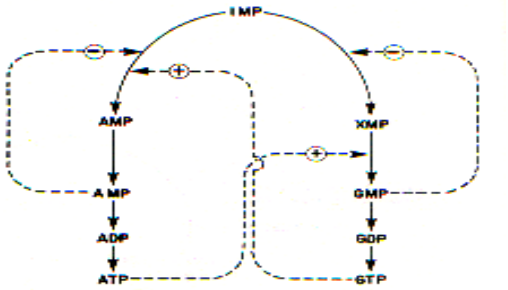
Ans. B. 8..

- Inosine monophosphate (IMP) is the parent nucleotide of purine from which both AMP and GMP are formed.
- Synthesis of IMP from the amphibolic intermediate such as glycine, glutamine, tetrahydrofolate derivatives, aspartate and ATP.
- The pathway then branches , one path leading from IMP to AMP, the other from IMP to GMP.
- Phosphoribosyl Pyrophosphate (PRPP) pool size regulates purine nucleotide biosynthesis.
- The major determinant of the overall rate of the de-novo purine nucleotide (AMP and GMP) biosynthesis is the concentration of PRPP.
- Concentration of PRPP reflects the relatives rate of PRPP synthesis , utilization and degradation.
- The enzyme PRPP synthase is sensitive both to phosphate concentration and to the purine ribonucleotides (AMP and GMP) that act as its allosteric regulators.



- Rate of PRPP synthesis depends on ribose-5-phosphate and on the activity of PRPP synthase.
- AMP and GMP feedback regulate their formation from IMP.
- AMP inhibits the enzyme adenylosuccinate synthase which involves the conversion of inosinate (IMP) into adenylosuccinate.
- Adenylosuccinate is a immediate precursor of AMP biosynthesis.
- GMP inhibits the enzyme IMP dehydrogenase which involves in conversion inosinate (IMP) into xanthylate (XMP).
- Xanthylate(XMP) is a immediate precursor of GMP biosynthesis.
- GTP is a substrate in the synthesis of AMP, whereas ATP is a substrate in the synthesis of GMP.
- This reciprocal substrate relation tends to balance the synthesis of adenine and guanine ribonucleotide.





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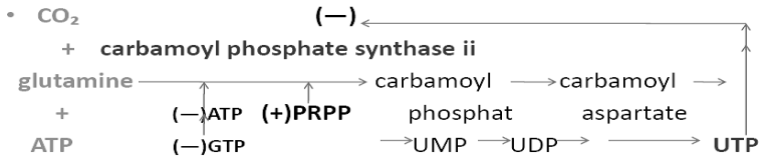
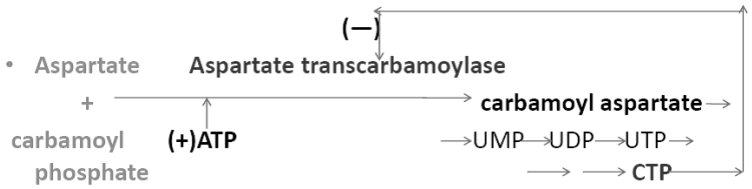
- Biosynthesis of pyrimidine (UMP and CMP)from the amphibolic intermediate such as PRPP, glutamine, carbon dioxide, aspartate, and for thymine nucleotides, tetrahydropholate derivatives.
- The activities of the first enzyme i.e, carbamoyl phosphate synthase ii of pyrimidine nucleotide biosynthesis is controlled by allosteric regulation.

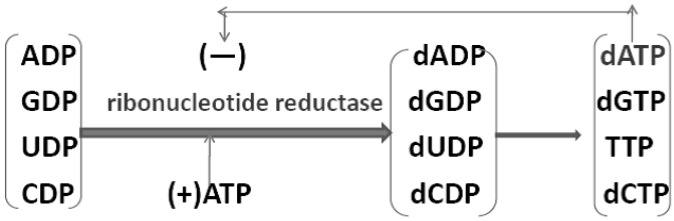


carbamoyl phosphate synthase ii is inhibited by UTP and purine nucleotide particularly ATP and GTP. carbamoyl phosphate synthase ii is activated by PRPP.

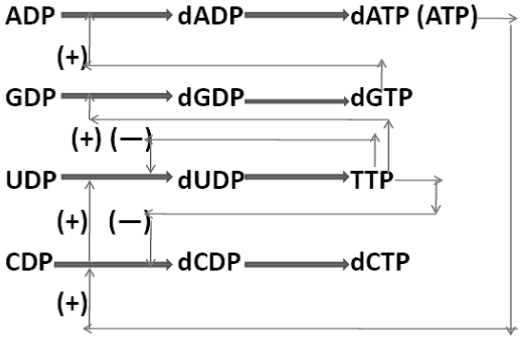
- The activity of the second enzyme i.e, Aspartate transcarbamoylase (ATCase) of pyrimidine nucleotide biosynthesis are controlled by allosteric regulation.



- Aspartate transcarbamoylase is inhibited by the CTP, the final product of pyrimidine biosynthesis and stimulated by ATP.
- The first three enzyme i.e, carbamoyl phosphate synthase ii,aspartate transcarbamoylase and dihydro-orotase and the last two enzymes i.e,OPRTase and OMP decarboxylase of the pyrimidine biosynthesis pathways are regulated by co-ordinate repression and derepression
- he synthesis of deoxyribonucleotides is controlled by the regulation of ribonucleotide reductase.
- The reduction of ribonucleotides to deoxyribonucleotides is precisely controlled by allosteric interactions.



- The overall catalytic activity of ribonucleotide reductase is diminished by the binding of dATP, which signals an abundance of deoxyribonucleotides
- The overall catalytic activity of ribonucleotide reductase is stimulated by the binding of ATP for the production of deoxyribonucleotides.
- The binding of dATP or ATP to the substrate specificity control site enhances the reduction of UDP and CDP to dUDP and dCDP repectively.



- The binding of thymidine triphosphate (TTP) promotes the reduction of GDP and inhibits the further reduction of pyrimidine ribonucleotides particularly UDP and CDP.
- The subsequent increase in the level of dGTP stimulates the reduction of ATP to dATP.
- This complex pattern of regulation supplies the appropriate balance of the four deoxyribonucleotides needed for the synthesis of DNA.
- Purine and pyrimidine nucleotide biosynthesis are co-ordinately regulated.
- Every mole of purine biosynthesis parallels with every mole of pyrimidine nucleotide biosynthesis.
- PRPP is synthesized by the enzyme PRPP synthase.
- PRPP forms a precursor essential for both purine and pyrimidine biosynthesis process.
- The synthesis of PRPP by the enzyme PRPP synthase is inhibited by feedback mechanism by both purine (AMP and GMP) and pyrimidine (UMP and CMP) nucleotides.
- There is requirement of ATP for CTP formation and there is stimulatory effect of GTP on CTP synthetase for CTP formation from UTP.
- By these effects ensure a balanced synthesis of purine and pyrimidine.