Unit1

Metabolic Pathways in Higher Plants and their Determination

PCI Syllabus

Metabolic pathways in higher plants and their determination

- Brief study of basic metabolic pathways and formation of different secondary metabolites through these pathways- Shikimic acid pathway, Acetate pathways and Amino acid pathway.
- Study of utilization of radioactive isotopes in the investigation of Biogenetic studies

Chapter Content

1.1 Brief Study of Basic Metabolic Pathways

Biogenesis or *in-vivo* synthesis of both primary and secondary metabolites starts with photosynthesis to produce sugar molecules which are metabolised to glycerates, pyruvates and finally acetyl CoA. This acetyl CoA is used in TCA cycle to generate a number of amino acids and excess is to synthesize fatty acids. Few of the acetyl CoA molecules are condensed to form mevalonic acid, precursor of synthesis of steroids and terpenoides. Amino acids give rise to alkaloids. The intermediates of glycolysis i.e. glyceraldehyde 3-phosphate and erythrose 4-phopshate from pentose phosphate pathway yields shikimic acid which is main precursor for biosynthesis of number of important aromatic chemicals like phenylpropanoides, lignin, lignans, flavonoides and terpenoid quinones.

1.1.1 Photosynthesis

Photosynthesis means "putting together with light". Photosynthesis in green plants and specialized bacteria is the process of utilizing light energy to synthesize organic compounds from carbon dioxide and water. Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green colour. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Carbon dioxide and oxygen enter and leave through tiny pores called *stomata*. It consists of the light dependent part (light reaction) and the light independent part (dark reaction, carbon fixation).

The general equation for photosynthesis is therefore:

$$2n\ CO_2 + 2n\ H_2O + \text{Sunlight} \rightarrow 2(CH_2O)n + n\ O_2 + 2n\ A$$

Carbon dioxide + Electron donor + Light energy → Carbohydrate + Oxygen + Oxidized electron donor

In the first stage, light-dependent reactions capture the energy of light and use it to make the energy-storage molecules ATP and NADPH via ATP synthase.

In the second stage, the light-independent reactions together known as *Calvin cycle*(Fig. 1.1) reduce carbon dioxide via enzyme RuBisCO (Ribulose-1, 5-bisphosphate carboxylase oxygenase). The product of the Calvin cycle is 3-carbon compound glyceraldehyde-3-phosphate and water. Two molecules of glyceraldehyde-3-phosphate combine to form one molecule of glucose and later different larger carbohydrates. The overall equation for the light-dependent reactions is:

$$2\ H_2O + 2\ NADP^+ + 2\ ADP + 2\ Pi + \text{Light} \rightarrow 2\ NADPH + 2\ H^+ + 2\ ATP + O_2$$

The overall equation for the light-independent reactions is :

$$3\ CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ Pi + 6\ NADP^+ + 3H_2O$$

In light independent part the carbon fixation refers to any process through which gaseous carbon dioxide is converted into a solid compound like sugar molecules. There are three types of Carbon fixation: C_3, C_4 and CAM. The difference between C_3 and C_4 photosynthesis depends on differences in the chemical compounds to which the incoming CO_2 is linked during the dark reactions (CAM photosynthesis differs from both C_3 and C_4 photosynthesis in that prior to fixation, CO2 is an acid form known as carbonic acid).

Differences between different Photosynthetic Pathways

C_3 pathway	C_4 pathway	CAM pathway
Calvin cycle or C_3 cycle or Calvin–Benson-Bassham cycle or CBB cycle or reductive pentose phosphate cycle is the most well-known type of photosyn-thesis.	Hatch and Slack's C4 photosynthesis.	Crassulacean Acid Metabolism (CAM) is daytime photosynthesis behind closed stomata through pre-fixed CO_2 during night.
First stable product is *a 3-carbon compound* called *phosphoglyceric acid*.	The first stable compound is a *4-C compounds oxalo acetic acid.*	the first stable compound is a *4-C compounds oxalo acetic acid*
Bundle sheath lacking chloroplasts	Mesophyll cells or bundle sheath having chloroplasts	Vacuoles in mesophyll cells
Enzyme Rubisco (*RuBP carboxylase)*	Pepco- enzyme (phosphoenol pyruvate *carboxylase)*	Pepco- enzyme (phosphoenol pyruvate *carboxylase)*
More efficient than C_4 or CAM photosynthesis when the environment is cool and water and light is plentiful.	Photosynthetically more efficient because the net requirement of ATP and $NADPH_2$ for the fixation of one molecule of CO_2 is considerably lower in C_4 plants than in C_3 plants.	Not efficient as C_3 or C_4 but advantage of CAM is that photosynthesis can proceed during the day while the stomata are closed, greatly reducing H_2O loss.
RuBP and CO_2 produces unstable 6-C Compound and gets cleaved to form two molecules of 3C compounds called phosphoglyceric acid (PGA).	During day times, oxaloacetate is converted into malate (malic acid), aspartate and then transported to inner compartment of bundle sheath where deacrboxylated to pyruvates.	During night, CAM plants through open stomata takes CO_2 and fixed it to organic acids stored in vacuoles. During the day CO_2 released to the Calvin cycle to build branched carbohydrates.
Requires more quantity of water hence optimum	Optimum temperature required is 30-40^oC.	as stomata remain shut during the photosynthesis

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temperature required is ***cool*** (20-25°C)		causes less water loss so useful for plants growing in ***hot***, dry day with cool nights or *stressful and arid conditions.*
Example- rice, wheat, potato	Example-maize, sugarcane	Example- pineapple, cacti, orchids

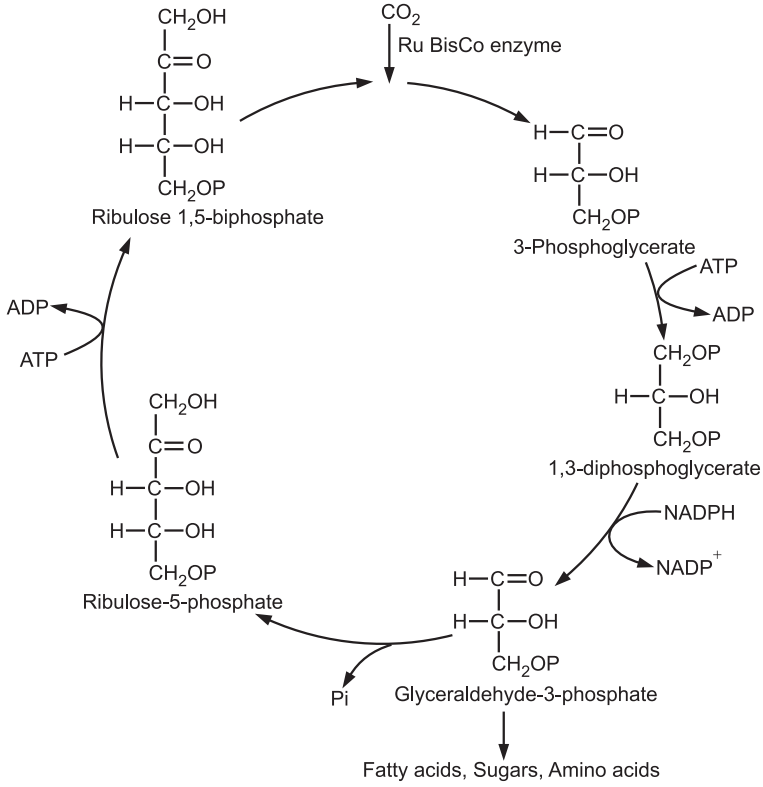


Fig.1.1 Calvin cycle

1.1.2 Glycolysis

Glycolysis (Fig.1.2) is the process of enzymatic reactions that convert glucose into three-carbon compounds, (pyruvate and glycerates) small amounts of ATP (energy) and NADH (reducing power). The glycolytic pathway operates in both situations,in the presence (aerobic) and absence (anaerobic) of oxygen. Under anaerobic conditions, the metabolism of each glucose molecule yields only two ATPs. In contrast, the complete aerobic metabolismof glucose to carbon dioxide by glycolysis and Krebs cycle yields up to thirty-eight ATPs. It is a central pathway that produces important precursor metabolites: six-carbon compounds of glucose-6P and fructose-6P and three-carbon compounds of glycerone-P, glyceraldehyde-3P, glycerate-3P, phosphoenolpyruvate and pyruvate. Acetyl-CoA, another important precursor metabolite, is

produced by oxidative decarboxylation of pyruvate in the presence of pyruvate dehydrogenase to form acetyl coenzyme A (acetyl CoA). Under conditions where energy is needed, acetyl CoA is metabolized by Krebs cycle to generate carbon dioxide and a large amount of ATP. When the cell does not need energy, acetyl CoA can be used to synthesize fats or amino acids.

As the glucose is oxidized by the glycolytic enzymes, the coenzyme nicotinamide adenine dinucleotide (NAD^+) is converted from its oxidized to reduced form (NADH). When oxygen is available (aerobic conditions), NADH can reoxidize to NAD^+. However, if either oxygen levels are insufficient (anaerobic conditions) or mitochondrial activity is absent, NADH must be reoxidized by the cell using some other mechanism. In animal cells, the reoxidation of NADH is accomplished by reducing pyruvate, the end-product of glycolysis, to form lactic acid. This process is known as *anaerobic glycolysis*. During vigorous exercise, skeletal muscles relie heavily on it. In yeast, anaerobic conditions result in the production of carbon dioxide and ethanol from pyruvate rather than lactic acid. This process, known as *alcoholic fermentation*, is the basis of wine production and the reason why bread dough rises.

Although some cells are highly dependent on glycolysis for the generation of ATP, the amount of ATP generated per glucose molecule is actually quite small. Therefore, in the majority of cells the most important function of glycolysis is to metabolize glucose to generate three-carbon compounds that can be utilized by other pathways. The final product of aerobic glycolysis is pyruvate.

1.1.3 Citric Acid Cycle

Citric acid cycle also known as the tricarboxylic acid (TCA) cycle or the Krebs cycle (Fig.1.3) is the common mode of oxidative degradation of carbohydrates, fatty acids and amino acids. The cycle starts with acetyl-CoA, the activated form of acetate, derived from glycolysis and pyruvate oxidation of carbohydrates and from beta oxidation of fatty acids. The two-carbon acetyl group in acetyl-CoA is transferred to the four-carbon compound of oxaloacetate to form the six-carbon compound of citrate. In a series of reactions two carbons in citrate are oxidized to CO_2 and the reaction pathway supplies NADH for use in the oxidative phosphorylation and other metabolic processes. The pathway also supplies important precursor metabolites including 2-oxoglutarate. At the end of the cycle the remaining four-carbon part is transformed back to oxaloacetate. Because two acetyl-CoA molecules are produced from each glucose molecule, two cycles are required per glucose molecule. Therefore, at the end of two cycles, the products are: 6 molecules of NADH, two molecules of $FADH_2$, two molecules of ATP, and four molecules of CO_2.

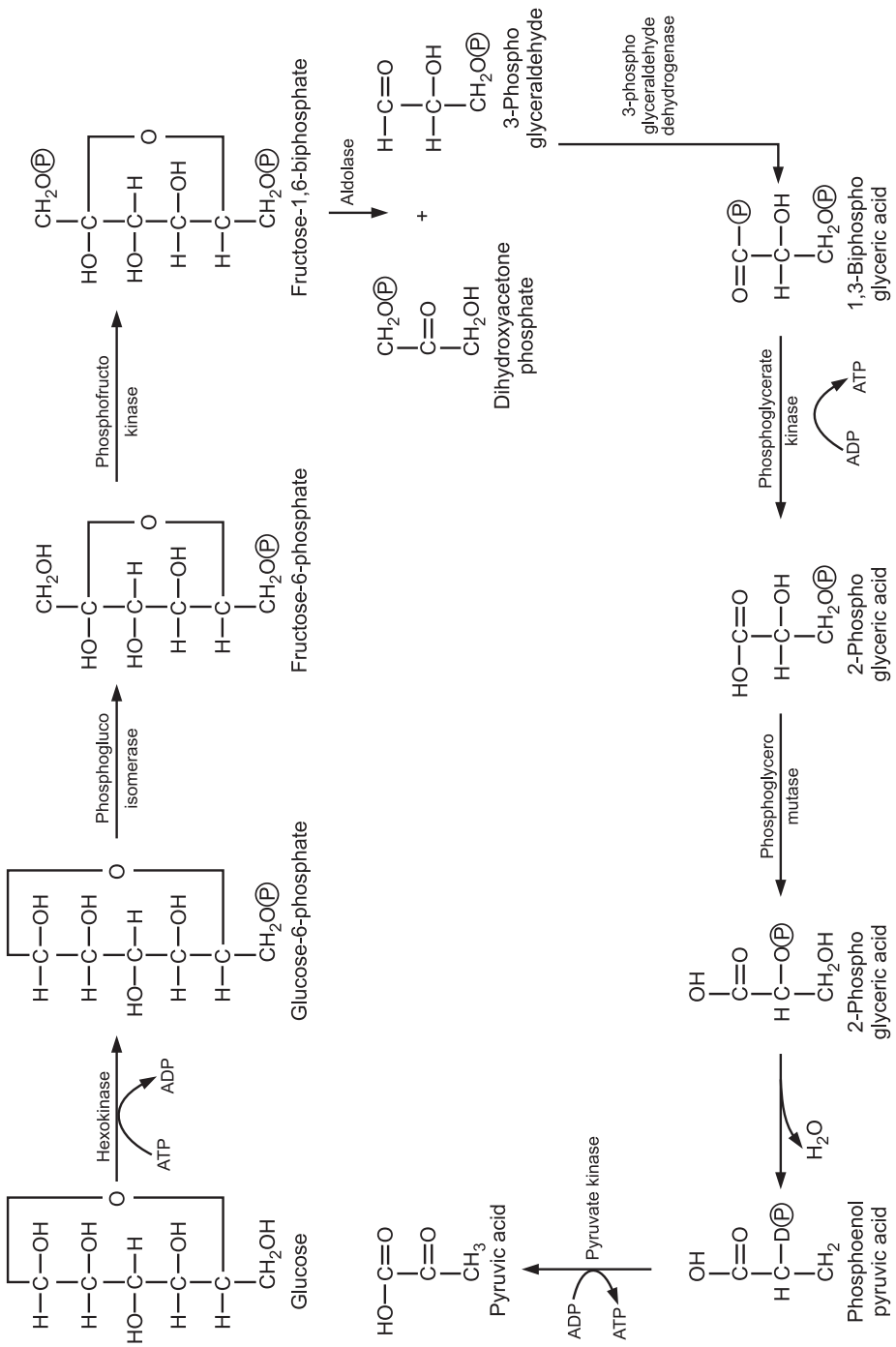


Fig.1.2 Glycolysis

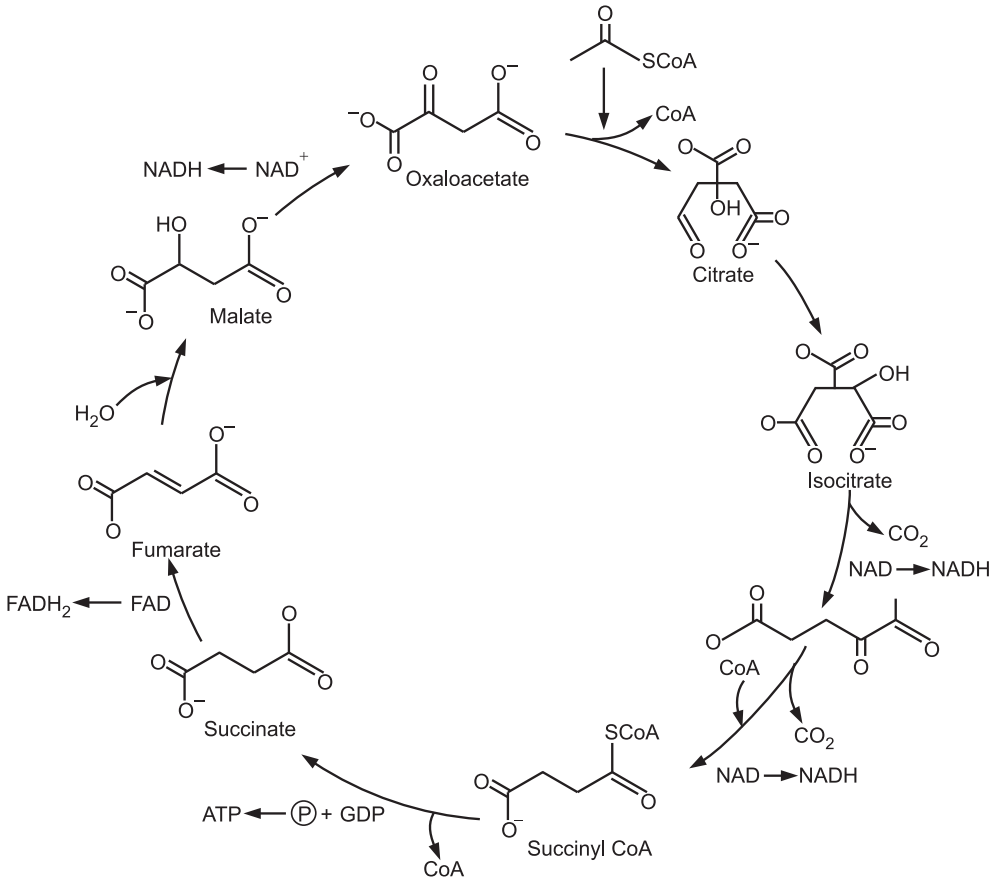


Fig.1.3 TCA Cycle

1.1.4 Pentose Phosphate Pathway

The pentose phosphate pathway (Fig.1.4), also called the phosphogluconate pathway or hexose monophosphate shunt, is a process that generates NADPH and 5-carbon sugars, pentoses. This pathway is an alternative to glycolysis. There are two distinct phases in the pathway. The first is the oxidative phase, in which NADPH is generated, and the second is the non-oxidative synthesis of pentoses. The role of this pathway can be summarized as:

- Production of NADPH,
- Production of ribose-5-phosphate used in the synthesis of nucleotides and nucleic acids.
- Production of erythrose-4-phosphate used in the shikimic acid pathway, synthesis of aromatic amino acids.

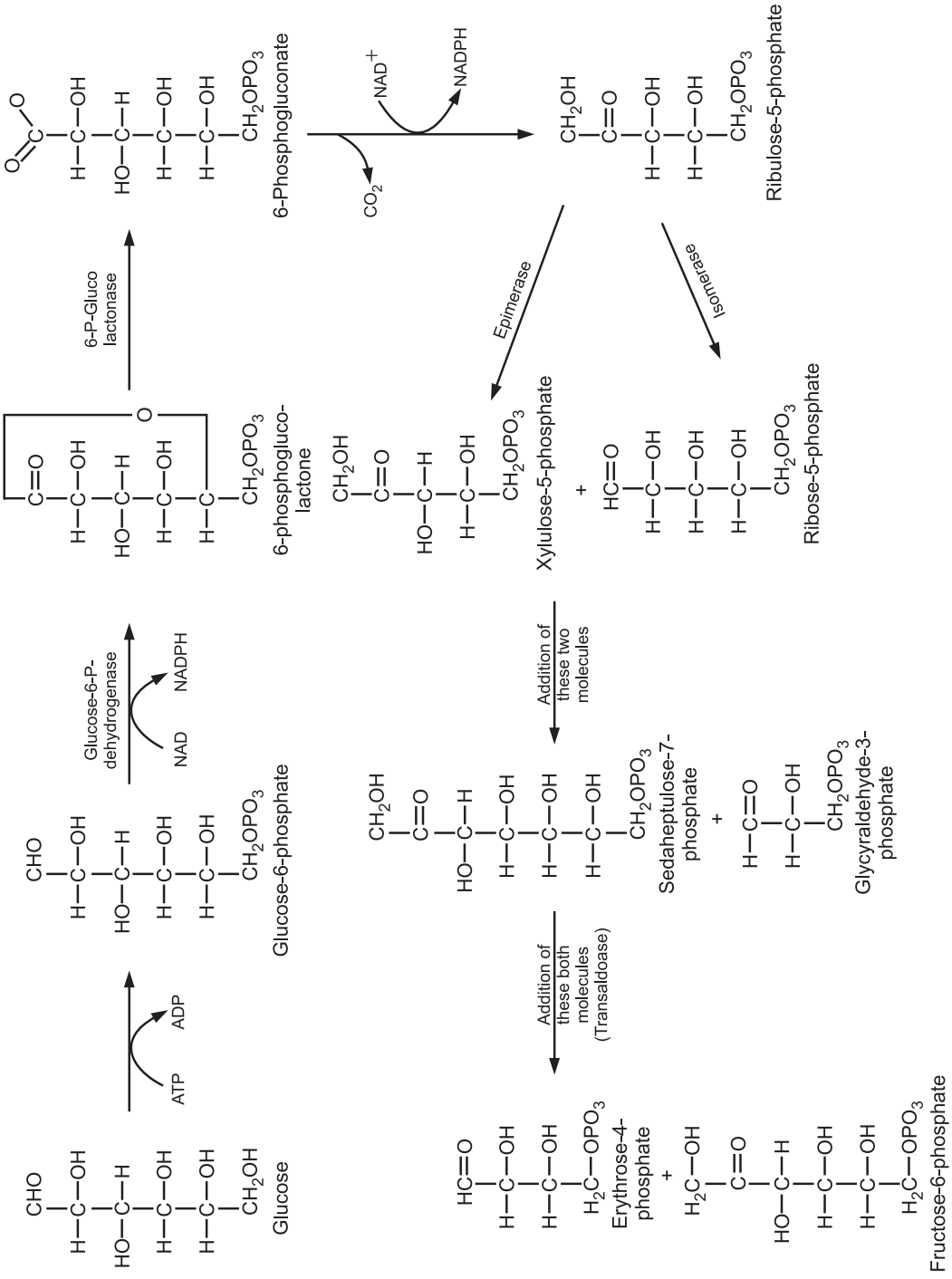


Fig.1.4 Pentose Phosphate Pathway

1.2 Acetate Pathway

1.2.1 Introduction

Acetate pathway is the pathway where acetate unit is the precursor for the biosynthesis of fatty acids and anthracene glycosides. In few compounds mevalonic acid or shikimic acid pathway intermediates are associated with acetate which further produces modified isoprenoidor flavonoid like compounds respectively.

Fatty acids are carboxylic acids with long hydrocarbon chains. The hydrocarbon chain length may vary from 10-30 carbons (most usual is 12-18). Fatty acids, also called as *aliphatic acids,* are important sources of energy stored in the form of triglycerides and act as intermediates in the biosynthesis of polyketides and hormones. Commercially, fatty acid and their derivatives are useful in the manufacturing of food, cosmetics and toiletries products such as soaps, papers, plastic, varnishes, paints and insecticides.

Fatty acids can be saturated and unsaturated depending on double bonds. Saturated fatty acids arealong-chain carboxylic acids that usually contain 12 and 24 carbon atoms with no double bonds. Unsaturated fatty acids which may be mono- or poly-unsaturated, are similar to saturated fatty acids, except that one or more alkenyl functional groups exist along the chain. Monounsaturated fatty acids (MUFAs) have only one double bond. Polyunsaturated fatty acids (PUFAs) have more than one double bond. Fatty acids are frequently represented by a notation such as C18:2 that indicate that the fatty acid consists of an 18-carbon chain and 2 double bonds.

Table 1.1 Fatty Acid ContainingPlants

Plant	Biological Source	Constituents	Uses
Almond	Seeds of *Prunusamygdalus*, Rosaceae	Oleic, linoleic, palmitic, stearic.	Adjuvant, emollient base
Arachis	Seeds of *Arachishypogaea*, Leguminosae	Arachidic, oleic, linoleic, palmitic, stearic	Emollient base
Bees wax	Honey comb of *Apismellifeca*, Apidae	Myristic, palmitic, cerotic	Ointment base
Carnauba	Leaves of *Coperniciaprunifera*, Palmae	Carnaubic, cerotic, melissyl	Opharmaceutical base
Castor	Seeds of *Ricinuscommunis*, Euphorbiaceae	Ricinoleic, oleic, linoleic, palmitic, stearic.	Purgative, emollient base
Chaulmoogra	Seeds of *Hydnocarpusheterophylla*, Flacourtricaceae	Chaulmoogric acid, hydnocarpic acid	Anti T. B., anilprotic
Coconut	Seeds kernel of *Cocosnucifera*, Arecaceae	Lauric, myristic, oleic, palmitic, stearic	Cosmetic preparation
Cod-liver	Fresh liver of *Gadusmorrhua*, Gadidae	Oleic, myristic, palmitic, stearic, DHA	Nutritive supplement
Corn	Grains of *Zeamays*, Gramineae	Oleic, linoleic, palmitic, stearic, arachidic, linolenic	Solvent for injection, cosmetic preparation

Table 1.1 *contd*…

Plant	Biological Source	Constituents	Uses
Cotton seed	Seeds of *Gossypiumhirsutum*, Malvaceae	Oleic, linoleic, palmitic, stearic	Cosmetic preparation
Evening primrose	Seeds of *Oenotherabiennis*, Onagraceae	Oleic, linoleic, palmitic, gamma linolenic.	Nutritive supplement
Jojoba	Seeds of *Simmondsiachinensis*, Buxaceae	Eicosenoic. Docosenoic, oleic acid	Cosmetic preparation
Karanja	Seeds of *Pongamiaglabra*, Papilionaceae	Oleic, linoleic, palmitic, stearic, arachidic, linolenic	Skin diseases
Kokum butter	Seeds of *Garciniaindica*, Guttiferae	Oleic, linoleic, palmitic, stearic, capric	Demulcent, emollient
Lard	Adnominal fat of *Susscrofa*, Suideae	Oleic, linoleic, palmitic, stearic.	Nutritive supplement
Linseed	Seeds of *Linum usitatissimum*, Linaceae	Oleic, linoleic, palmitic, stearic.	Edible oil, demulcent
Mustard	Seeds of *Brassica nigra*, Cruciferae	Arachidic, linolenic, linoleic, oleic, myristic	Counter irritant, rubefacient.
Olive	Fruits of *Olea europaea,* Oleaceae	Oleic, linoleic, palmitic, stearic	Edible oil, emollient base
Palm kernel	Kernel of *Elaeisguineensis,* Arecaceae	Oleic, linoleic, palmitic, stearic, lauric, myristic	Cosmetic toiliteries preparation
Rapeseed	Seeds of *Brassica napus,* Cruciferae	Oleic, linoleic, palmitic, stearic, erucic, alpha linolenic	Edible oil
Rice bran	Seeds of *Oryza sativa,* Gramineae	Oleic, linoleic, palmitic, stearic	Edible oil
Safflower	Seeds of *Carthamus tinctorius,* Compositae	Oleic, linoleic, palmitic, stearic, arachidic, linolenic	Edible oil
Sesame	Seeds of *Sesamum indicum,* Pedaliaceae	Oleic, linoleic, palmitic, stearic	Edible oil, cosmetic toiliteries preparation
Shark liver	Fresh liver of *Hypoprion*brevirostris,	DHA (docosahexaeonic acid) and EPA (eicosapentaenoic acid).	Antixeropthalmic factor
Soya bean	Seeds of *Glycine max,* Leguminosae	Oleic, linoleic, palmitic, stearic, alpha linolenic	Edible oil
Spermaceti	Head of *Physeter macrocephalus,* Physeteridae	Lauric, myristic, stearic, cetyl palmitate	Ointment base
Suet	Abdominal fat from *Oviesaries,* Bovidae	Oleic, myristic, palmitic, stearic	Nutritive supplement
Theobroma	Kernels of *Theobroma cacao,* Sterculiaceae	Oleic, linoleic, palmitic, stearic	Suppository base
Wheat germ	Wheat germs of *Triticum aestivum,* Gramineae	Oleic, linoleic, linolenic	Nutritive supplement
Sunflower	Seeds of *Helianthus annuus,* Compositae	Oleic, linoleic, palmitic, stearic	Edible oil

Table 1.2 Saturated Fatty Acids Examples

Common Name	Structural Formula
Propionic acid	CH_3CH_2COOH
Butyric acid	$CH_3(CH_2)_2COOH$
Valeric acid	$CH_3(CH_2)_3COOH$
Enanthic acid	$CH_3(CH_2)_5COOH$
Caprylic acid	$CH_3(CH_2)_6COOH$
Capric acid	$CH_3(CH_2)_8COOH$
Lauric acid	$CH_3(CH_2)_{10}COOH$
Myristic acid	$CH_3(CH_2)_{12}COOH$
Palmitic acid	$CH_3(CH_2)_{14}COOH$
Margaric acid	$CH_3(CH_2)_{15}COOH$
Stearic acid	$CH_3(CH_2)_{16}COOH$
Arachidic acid	$CH_3(CH_2)_{18}COOH$
Behenic acid	$CH_3(CH_2)_{20}COOH$
Tricosylic acid	$CH_3(CH_2)_{21}COOH$

Table 1.3 Unsaturated Fatty Acids Examples

Common Name	Chemical Structure
Myristoleic acid	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$
Palmitoleic acid	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
α-Linolenic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
Arachidonic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
Eicosapentaenoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
Erucic acid	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$
Docosahexaenoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOH$

1.2.2 Saturated Fatty Acid Biosynthesis

Saturated fatty acids are synthesized by a series of decarboxylative Claisen condensation reactions from acetyl-CoA and malonyl-CoA in the presence of enzyme fatty acid synthase. Enzyme synthase contains ACP as part of its structure. Following each step of elongation the β-keto group is reduced to the fully saturated carbon chain by the sequential action of enzymes − ketoreductase, dehydratase and enol reductase. Fatty acid synthesis (Fig.1.5) starts with acetyl CoA which is the two carbon containing precursor. This is used to add two carbons to growing fatty acid chain stepwise. This explains why fatty acids always have an even number of carbons. This process occurs in the cytosol.

During biosynthesis, the growing fatty acid chain gets attached covalently to the phosphopantethiene prosthetic group of ACP (acyl carrier protein) which allows intermediates to remain covalently linked to the synthases and access this intermediates to the right enzyme-active sites. Acyl Carrier Protein (ACP), converts malonyl CoA to malonyl ACP. ACP synthase is an enzyme that holds the growing fatty acid chain. Acyl enzyme thioester releases C_2 unit to malonyl ACP to form fatty acyl ACP through sequence of reduction, dehydration reactions. This fatty acyl ACP on attack of water generates fatty acids. Triglycerides (esters of glycerol containing same or different 3 fatty acids) are biosynthesized from glycerol 3-P (product of Calvin cycle) and first fatty acyl CoA esterification process which is elaborated in Fig.1.5.

Fatty acid synthesis is simply a linear combination of acetate units facilitated by enzyme fatty acid synthase. ACP allows growing fatty acid chain to react with thio group of enzyme fatty acid synthase and thus head to tail condensation followed by reduction gives rise to a long chain of saturated fatty acid. Mostly even numbers of carbon containing fatty acids are common in nature. But when starting compound is other than acetate (example- propionic acid), odd number of C-containing fatty acids can also be synthesized by plants. C_{16} and C_{18} (Palmitic and stearic acids respectively) are the most common saturated fatty acids.

In fatty acid biosynthesis, acetate is the starter group and malonate is a chain extender. But in a few compounds there may be change in starter or chain extender group. Cinnamoyl CoA obtained from shikimic acid pathway acts as a starter group in the synthesis of flavonoid and stilbenes. Anthranilolyl CoA obtained from anthranilic acid is used in the synthesis of quinoline and acridine alkaloids. Hexanoate is the starter group in the formation of aflatoxins and cannabinoides. Incorporation of propionate as a chain extender other than mevalonate from propionyl CoA or methyl malonyl CoA leads to the formation of macrolide antibiotics.

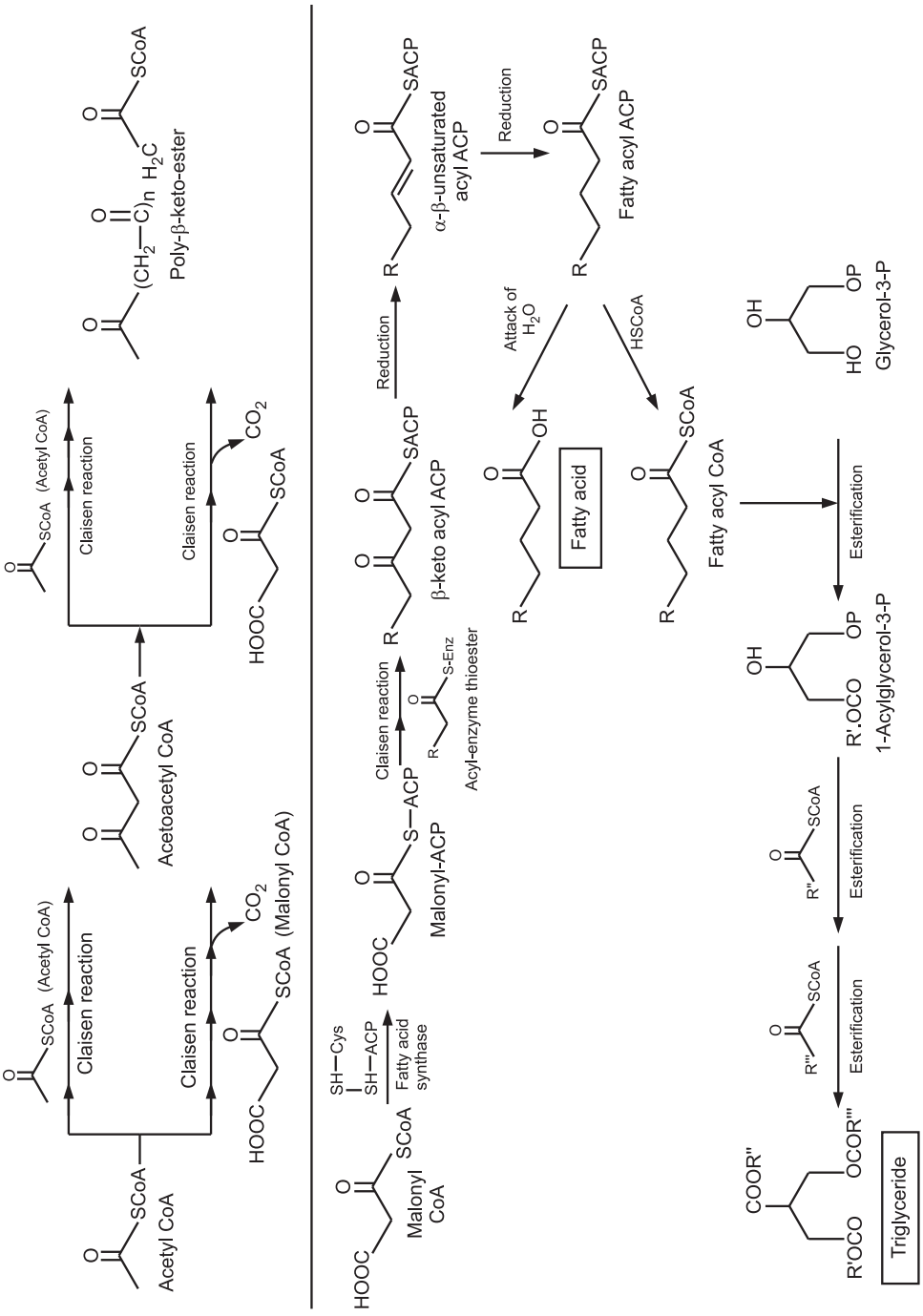


Fig.1.5 Acetate Pathway

1.2.3 Unsaturated Fatty Acid (UFA) Biosynthesis

UFAs are synthesized by sequential desaturation mechanism. Double bond is introduced by enzyme desaturase, oxidation and NADPH or NADH. Stearic acid is the common starting material to give various UFAs.

Essential fatty acids belong to the class of PUFAs. There are two types of essential fatty acids; omega-6 and omega-3 fatty acids which can be short chain (omega-3α linolenic acid, omega-6 eicosapentaenoic acid or EPA, docosahexaenoic acid DHA) or long-chain polyunsaturated fatty acids (omega-3α Linoleic acid, omega-6α gamma-linolenic acid or GLA, dihomo-gamma-linolenic acid or DGLA, arachidonic acid or AA). Linoleic acid and isomers of linolenic acid are EFA. As its derivative linolenate blocks synthesis of prostaglandins and hence useful in the treatment of various ailments especially cardiovascular diseases. Linoleic acid found to be the important compound in the prevention of hair loss, cancer, cystic fibrosis and dermatitis. DHA is present in mothers's milk and constituent of brain and retina. The consumption of alpha linolenic acid can be converted to DHA by animals which is used to prevent brain related diseases, colon cancer and cardiovascular risks.

In animals, EFA are obtained from diet whichact as precursors for synthesis of DHA, which is (Fig.1.6) important (component) for normal functioning of brain. Dihomolinoleic acid (PGE_1), arachdinoic acid (PGE_2) and EPA (PGE_3) are important precursors for biosynthesis of prostaglandins. PGH_2 synthesizes thromboxanes and arachidonic acid syntehsizes leukotrienes. Eicosanoid is the collective term for oxygenated derivatives of three different 20-carbon essential fatty acids i.e.eicosapentaenoic acid (EPA), arachidonic acid (AA) and dihomo-gamma-linolenic acid (DGLA). There are four types of eicosanoids i.e. prostaglandins, prostacyclins, thromboxanes and leukotrienes.

Various branched chain fatty acids are biosynthesized by various mechanisms. Sterculi, malvalic acid and chaulmoogric acid are some of the important branched chain fatty acids obtained from stearic acid by alkylation, desaturation (sterculic) oxidation (malvalic). 2-cyclopentenyl carboxyl CoA as a starter unit and malonyl CoA as an extender unit gives chaulmoogric acid.

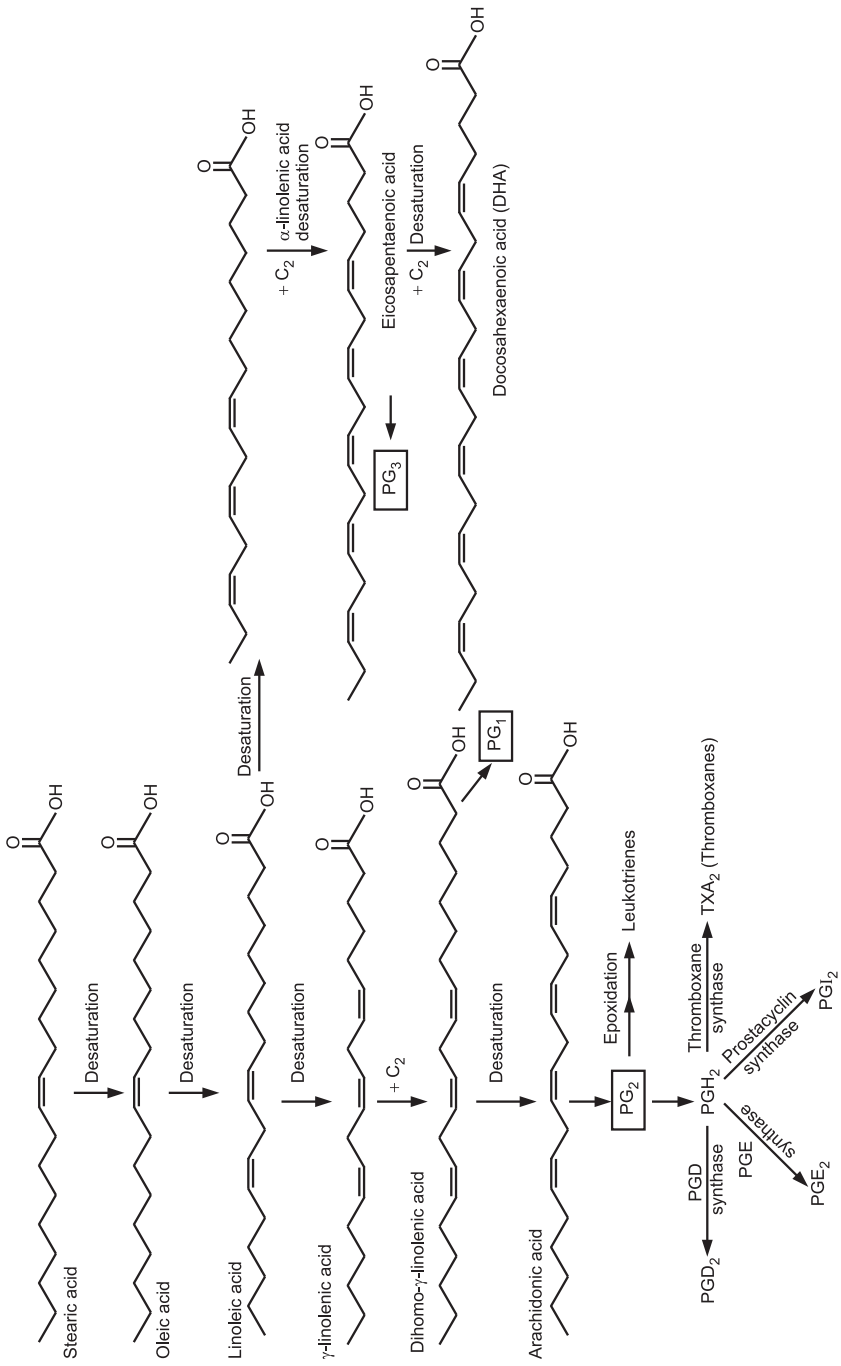


Fig.1.6 Biosynthesis of Prostaglandins, thromboxanes and Leukotrienes - an overview and PUFA

1.3 Shikimic Acid Pathways

Shikimic acid pathway is significantly important as it leads to formation of almost all aromatic compounds present in nature like phenylpropanoides (lignans, lignnins), coumarin, flavonoids and isoflavonoids and terpenoid quinones.

Shikimate pathway (Fig.1.7) starts with erythrose 4-phospate (obtained from the pentose phosphate pathway) and phosphoenolpyruvate (obtained from glycolysis pathway) coupling to yield phosphorylated 7-carbon keto sugar, 3-deoxy-D-arabino-heptulosonic acid-7-phosphate, (DHAP). DHAP on removal of phosphoric acid cyclizes to 3-dehydroquinic acid which on reduction yields quinic acid. By dehydration3-dehydroquinic acid forms 3 dehydroshikimic acid which forms shikimic acid followed by reduction.

3-dehydroshikimic acid on dehydration and enolisation forms protocatechuic acid and on dehydrogenation and enolisation forms gallic acid which is the component of many types of tannins.

Shikimic acid through phosphorylation and elimination reactions forms very important intermediate chorismic acid. Chorismic acid via simple rearrangement gives prephenic acid. Chorismic acid on another branch by glutamine mediated amination at C_2 gives anthranilic acid while amination at C_4 position (Fig. 1.8) gives P-aminobenzoic acid (PABA). PABA is a part of folic acid structure. Prephenic acid on dehydration and decarboxylation yields precursor of phenylalanine i.e.phenylpyruvic acid. On dehydrogenation and decarboxylation, prephenic acid yields p-hydroxy phenylpyruvic acid which is direct precursor of tyrosine.

Isochorismic acid, isomer of chorismic acid, on pyruvic acid elimination forms salicylic acid known as phenolic phytohormone present in *Salix alba*, Salicaceae and *Filipendulaulmaria*, Rosaceae. (Fig.1.8).

Phenylalanine on enzymatic deamination forms cinnamic acid which is the starting material for biosynthesis of various phenylpropanoids. Phenylpropanoid compounds are so named because of the basic structure of a three-carbon side chain on an aromatic ring, which is derived from L-phenylalanine.

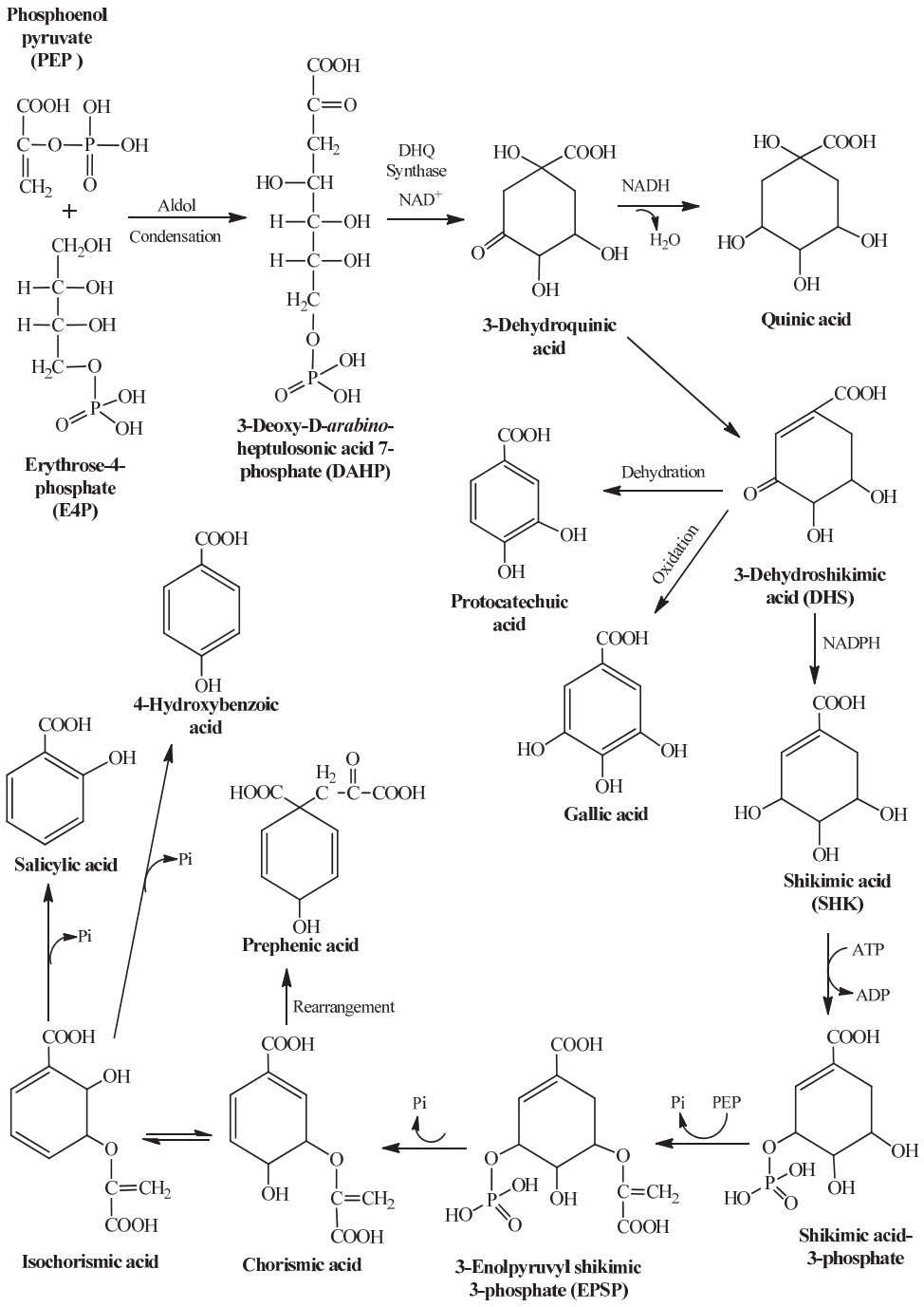


Fig.1.7 Shikimic acid Pathway Part-I

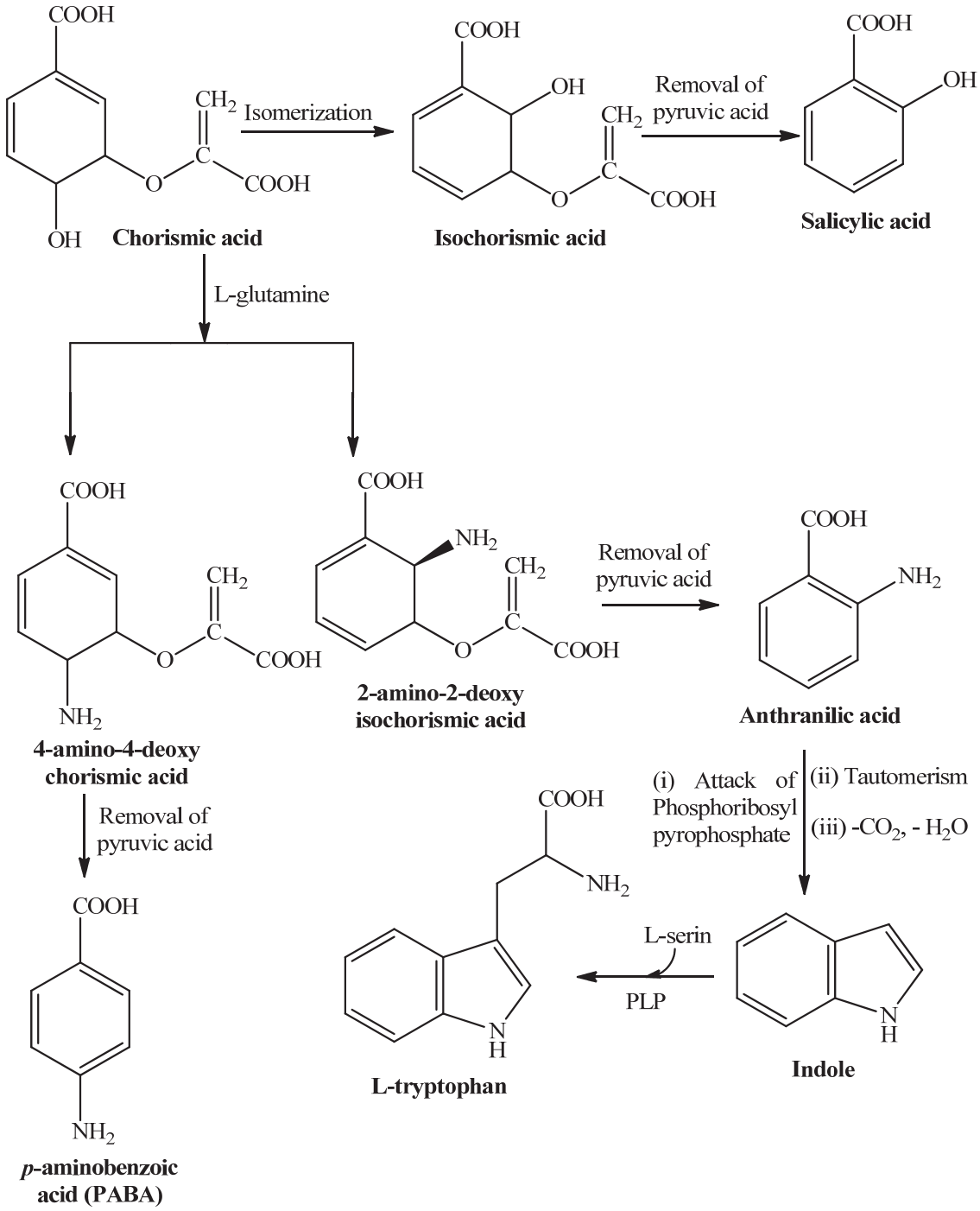


Fig.1.8 Shikimic acid Pathway–Part-II

1.4 Amino Acid Biosynthesis Pathway

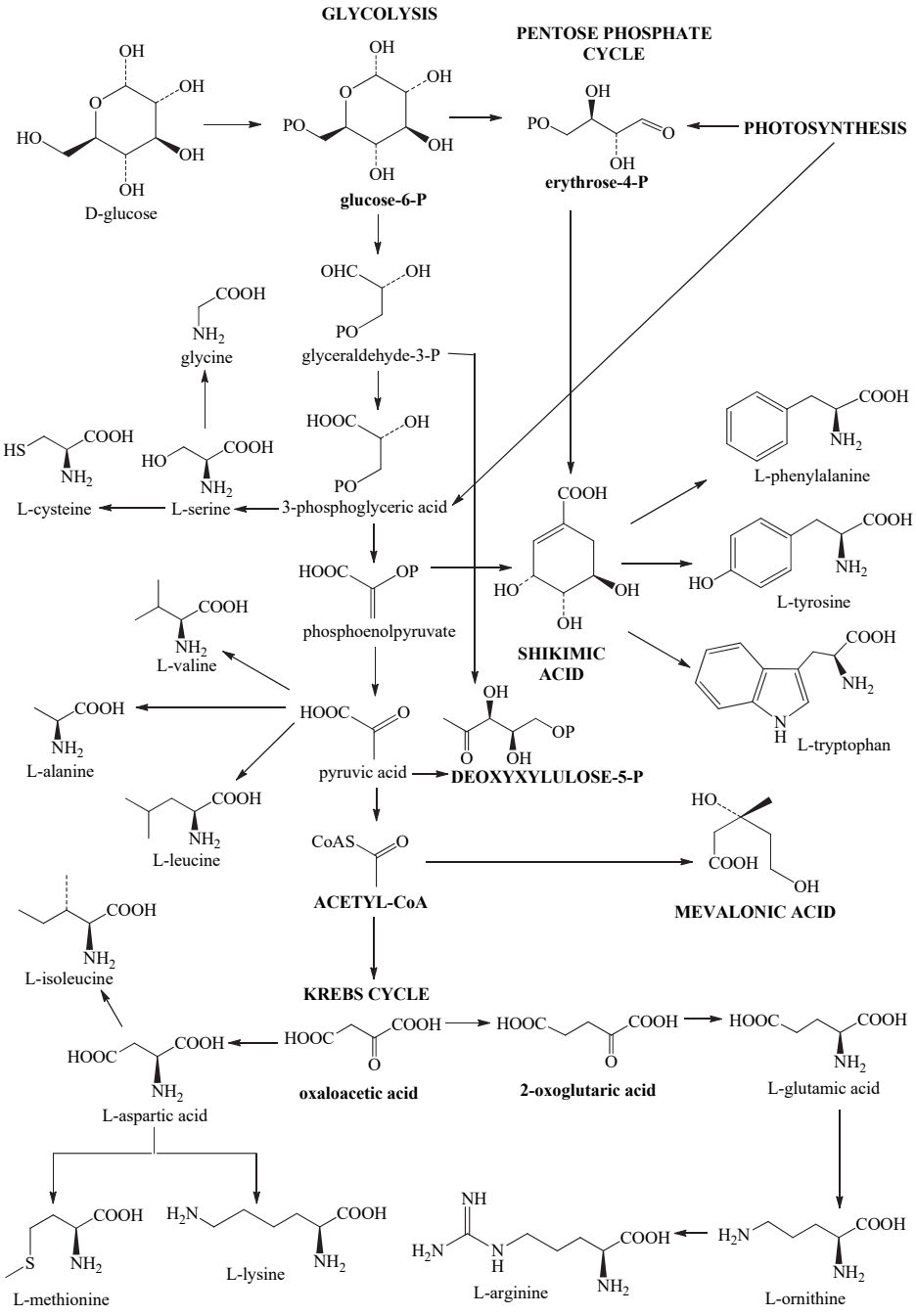
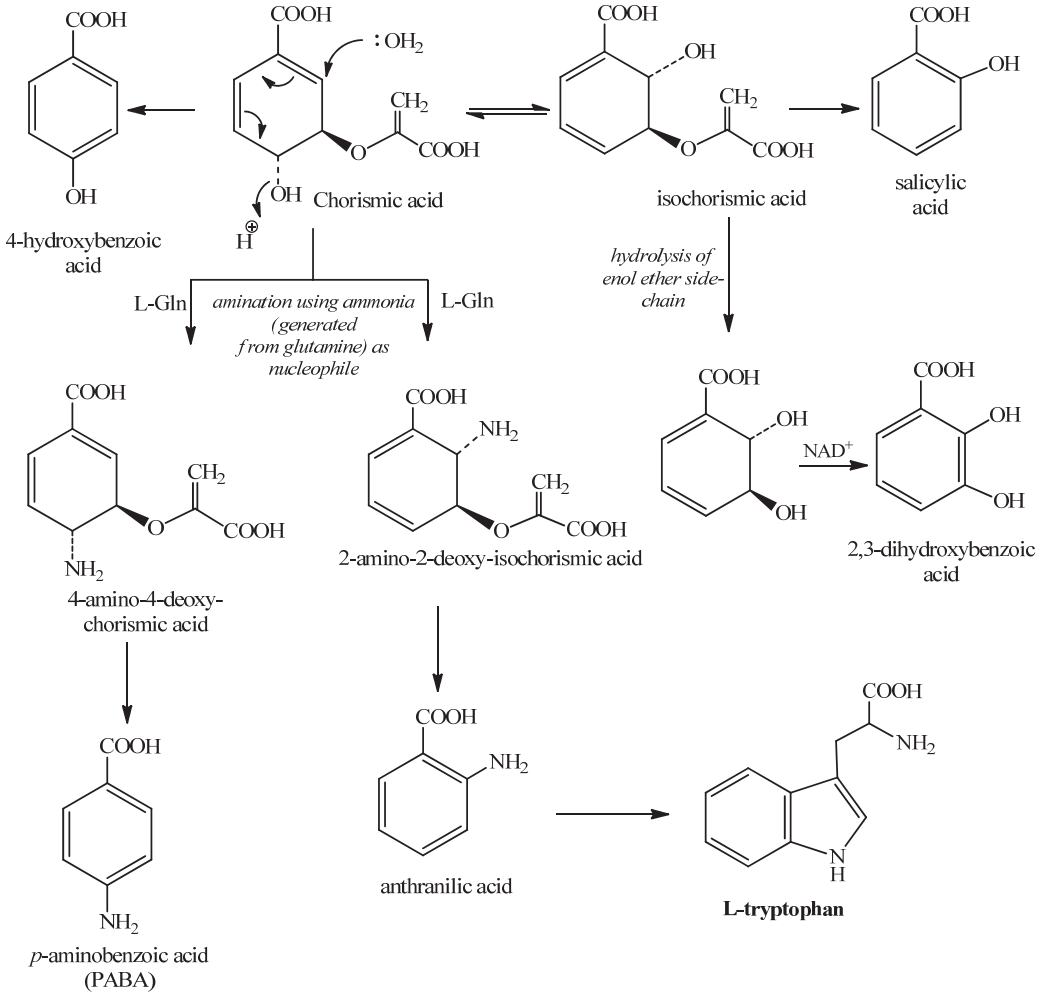


Fig.1.9 General scheme of Amino acid biosynthesis

- All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway (Fig.1.9).
- Nitrogen enters these pathways by way of glutamate and glutamine. Some pathways are simple, others are not.Biosynthesis of serine, glycine, cysteine, homocysteine, methionine, proline, orinithine, arginine are outlined in Fig. 1.10 to 1.14.
- Ten of the amino acids are only one or a few enzymatic steps removed from their precursors. The pathways for others, such as the aromatic amino acids, are more complex.
- Different organisms vary greatly in their ability to synthesize the 20 amino acids. Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them



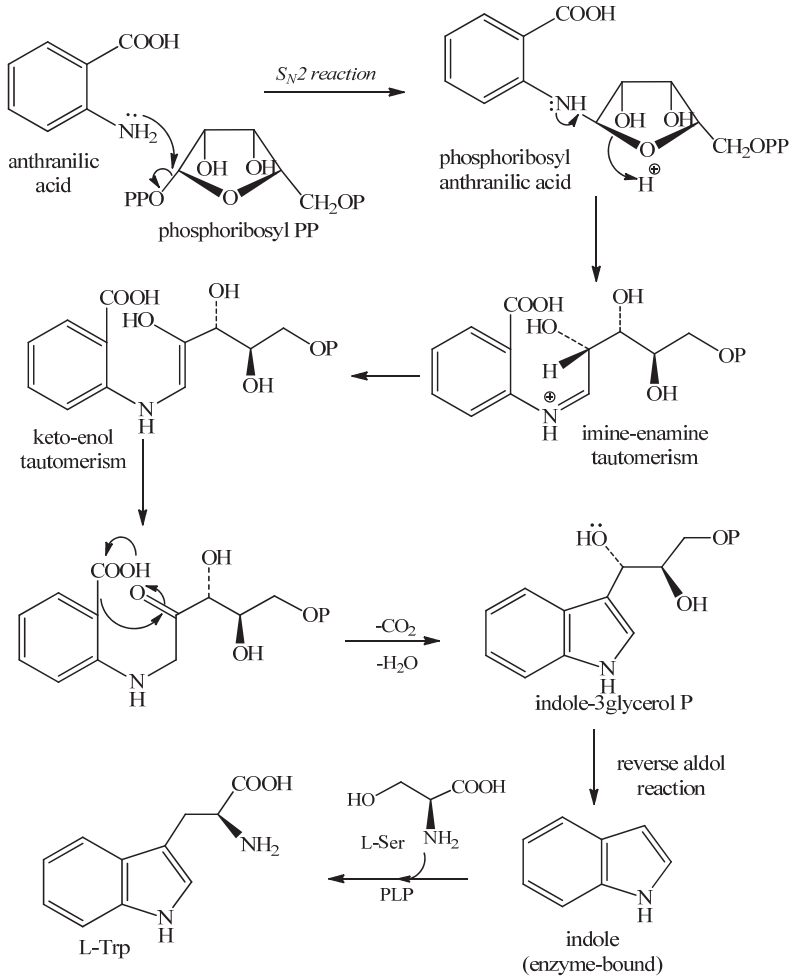


Fig.1.10 Biosynthesis of Tryptophan

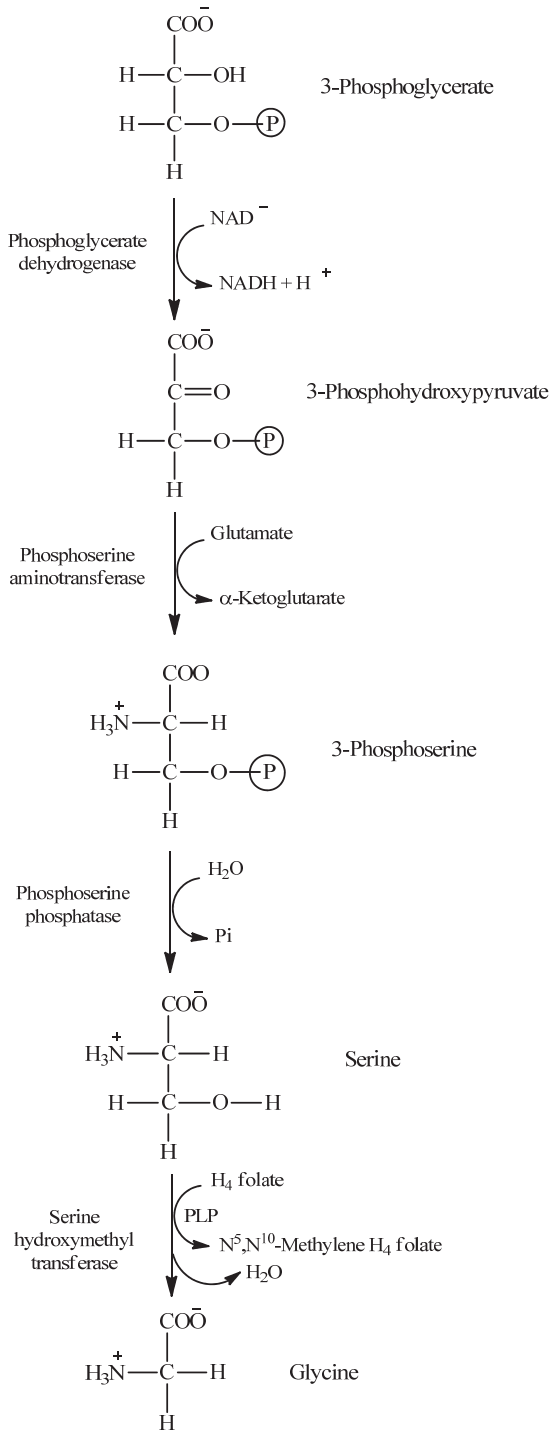


Fig.1.11 Biosynthesis of Serine, Glycine

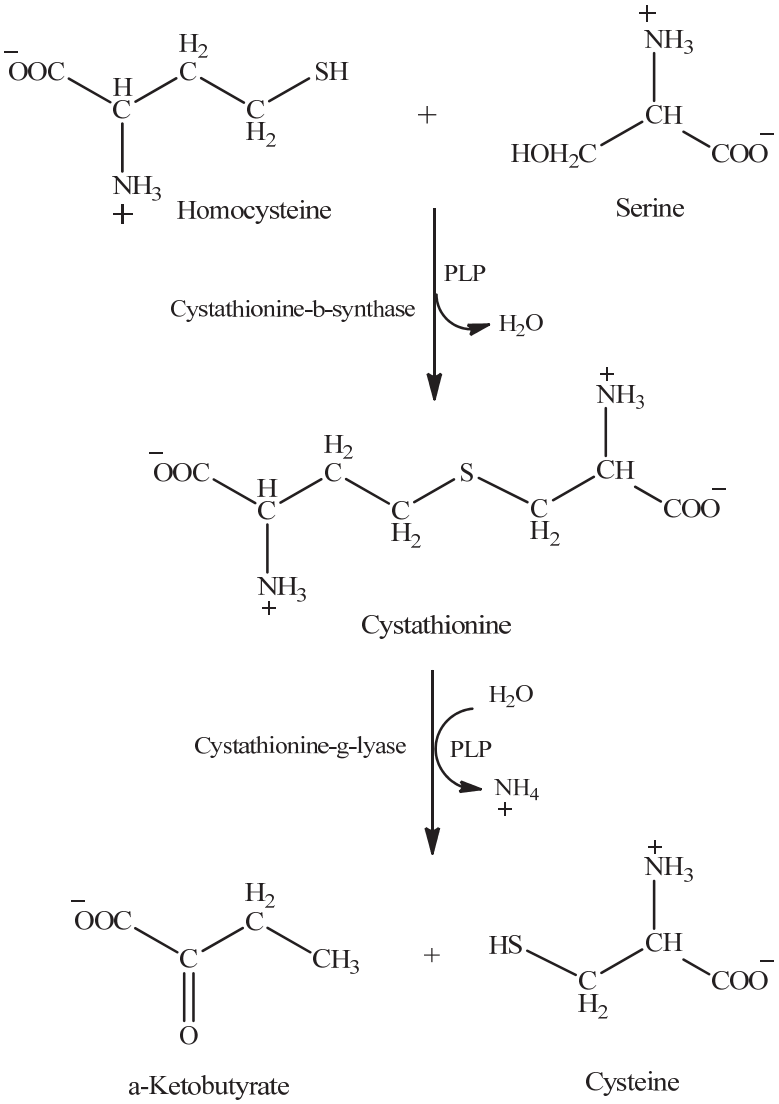


Fig.1.12 Biosynthesis of Cysteine

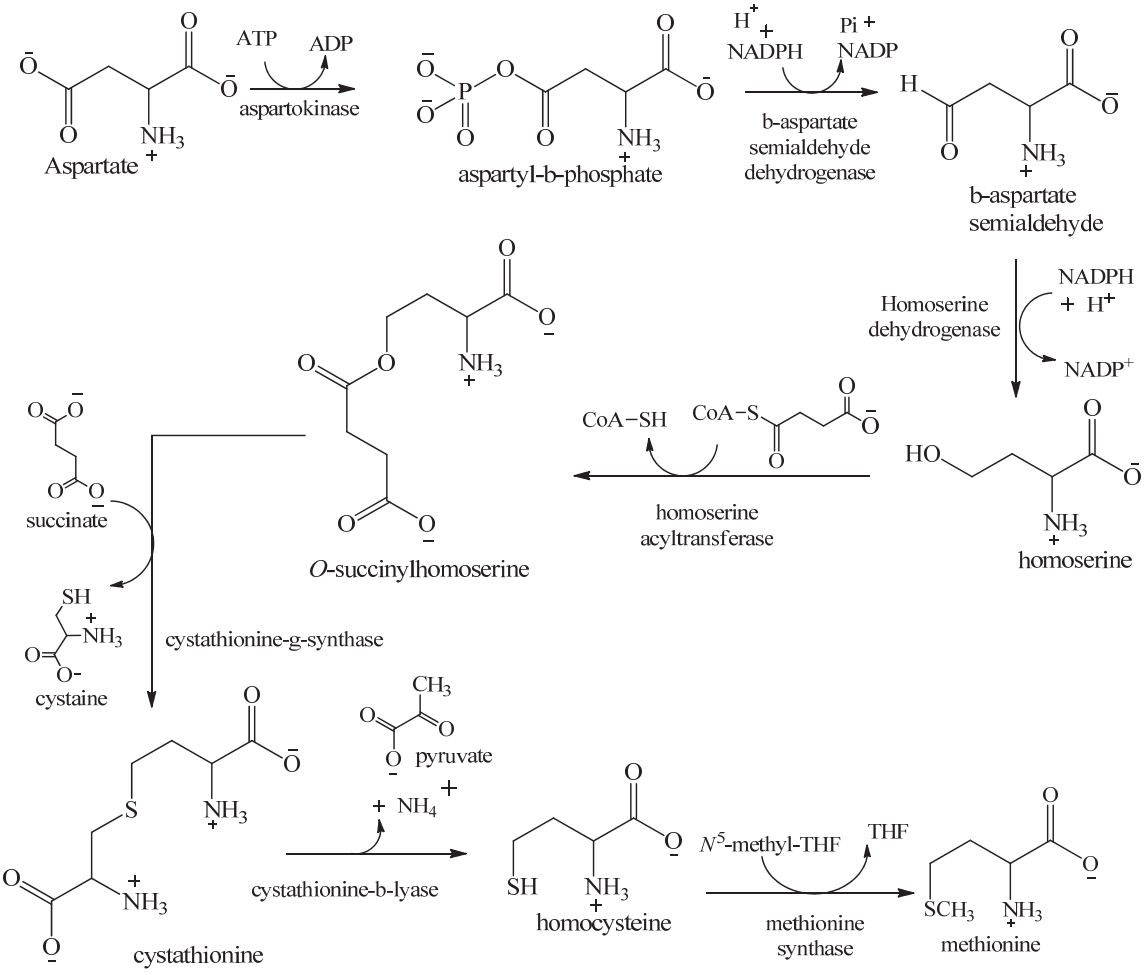


Fig.1.13 Biosynthesis of homocysteine and methionine

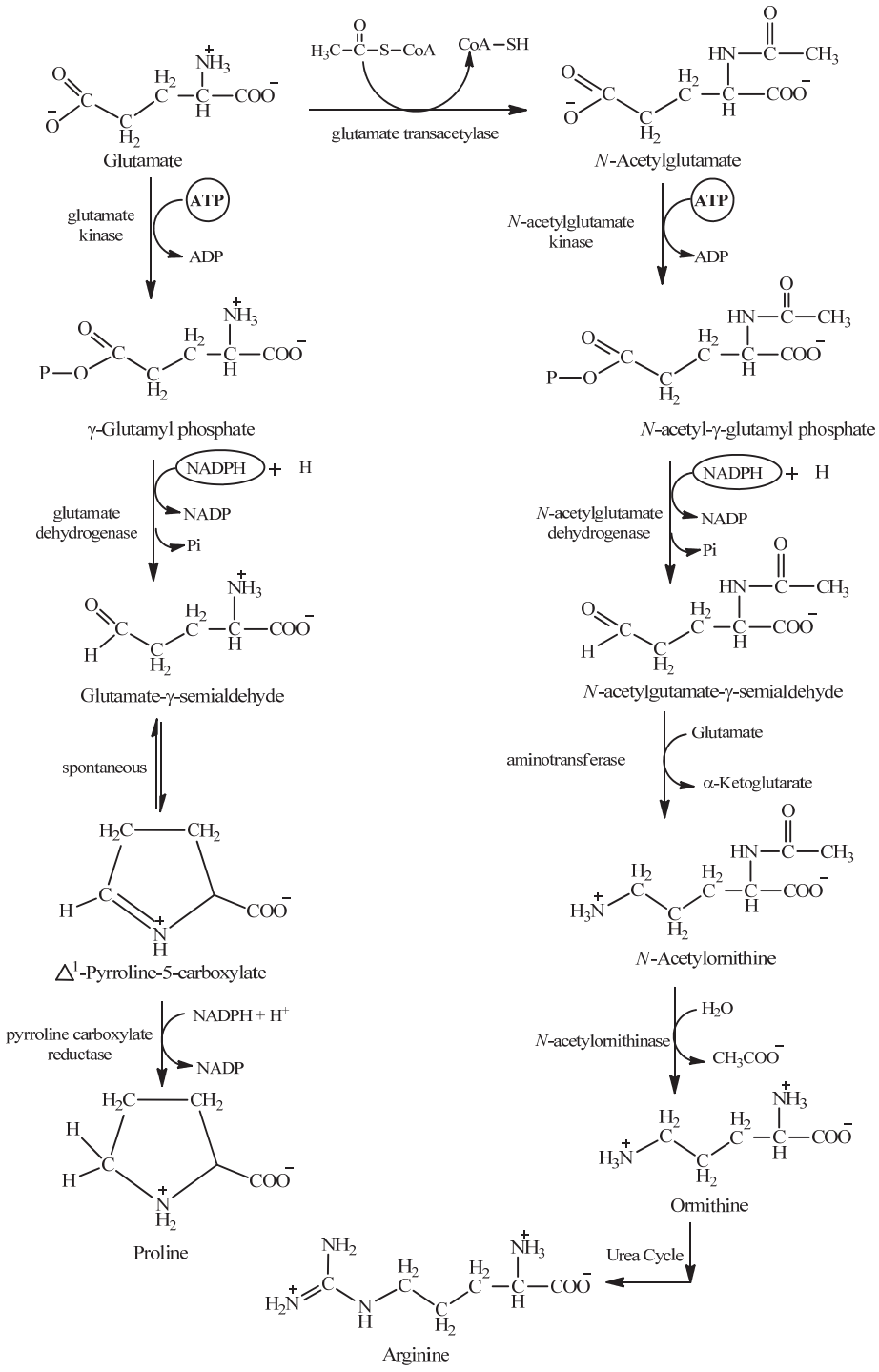


Fig.1.14 Biosynthesis of Proline, Orinithine, Arginine

1.5 Elucidation of Biosynthetic Pathway

After discussing the above things now one question will certainly rise that how this pathways of secondary metabolite biosynthesis are elucidated? Actually the elucidation of pathway is very first step of biosynthesis study. New advances in chemistry and analytical techniques have made possible to explore various biosynthetic pathways with a very clear picture of precursors, intermediates, products, enzymes and reactions involved.

Techniques of Elucidation of Biosynthetic Pathway

Technique	Details
Use of isolated organ	It is useful to locate site of biosynthesis of particular compounds. This is Tissue culture technique useful to determine site of synthesis as well as whole pathway through addition of radioactive tracers in cultures of plants parts. Example: *Datura* and *Nicotinana* species
Grafting method	It is also useful to locate site of biosynthesis of particular compounds. In this method, cut portion of one plant (scion) is grafted on major plant (stock) to identify and confirm exact site of synthesis of secondary metabolites. Example: scion of datura plant and stock of tomato plant showed less accumulation of tropane alkaloids while stock of datura plant and scion of tomato plant increased amount of alkaloids.
Mutant strain use	It is useful to confirm intermediates and enzymes responsible for biosynthesis of particular compounds. Natural or induced mutation in lower plants or fungi useful to determine and confirm intermediates or product due to blockages of pathway. Many times one of the intermediates get accumulated and thus whole biosynthesis stopped that time artificial supply of enzymes or intermediate helps in normalizing pathway.
Enzyme studies	It is useful to confirm enzymes responsible for reactions. Various enzymes can be isolated and studied for their exact role in different biosynthetic reactions by traditional biochemical methods.
Genomic studies	It is useful to study chemical reactions involved in biosynthesis of metabolites via responsible proteins and or related DNA and genes by simple biochemical studies or modern genetic analysis
Tracer technique	It involves use of radioactive and stable isotopes to elucidate biosynthetic pathways. Details are as follows:

Study of utilization of radioactive isotopes in the investigation of Biogenetic studies

(Tracer Technique)

Use of radio labelled tracers is the most preferred technique of elucidation due to its specificity and selectivity. Tracer technique is also useful to confirm the exact precursor or intermediate from the number of possible precursors or intermediates. The whole sequence can also be generated by use of tracer technique. It involves the following steps:

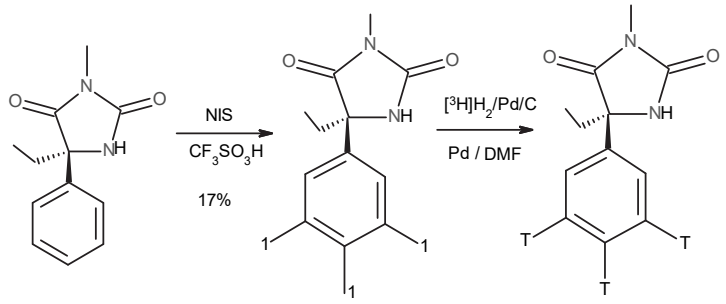
Step-1 Selection of suitable radioisotope: The selection of radioisotope depends on half-life so that it should withstand the long and unpredictable time period of biosynthesis. ^{14}C and ^{3}H are

the most commonly used radio isotopes. Now-a-days, stable isotopes like ^{13}C are also utilized to elucidate biosynthesis with the help of sophisticated NMR analysis. Stable isotope labelling involves the use of non-radioactive isotopes that can act as a tracers used to model several chemical and biochemical systems. The chosen isotope can act as a label on that compound that can be identified through nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Properties of radioactive and stable isotopes		
Radioactive isotope	**Radiation**	**Half-life**
Carbon (C^{14})	Beta	5760 years
Hydrogen (H^3)	Beta	12.5 years
Sulphur (S^{35})	Beta	871 days
Phosphorus (P32)	Beta	14.3 days
Chlorine (Cl^{36})	Beta	4.4×10^5 years
Iodine (I^{131})	Beta, gamma	8 days
Cobalt (Co^{60})	Beta, gamma	5.3 days

Step-2 Labelling of precursor or intermediate: The labelling of any metabolite by radioisotope is a tedious and complex procedure involving many chemical steps

Step-3 Insertion of radio-labelled metabolite in plant part: After labelling the desired metabolite, the next step is to introduce it into the suitable organ of a plant (e.g. root, stem, leaves) by proper method (e.g. immersion, injection and spraying). Tissue cultures of plant parts are also preferred for introduction of labelled metabolite/s. The whole pathway elucidation as well as the exact site of biosynthesis can be studied by tissue culture. Intact roots can be grown in tracer solution. Even the plant can be grown in an atmosphere containing $^{14}CO_2$ or $^{13}CO_2$ tracers. An example of palladium-catalyzed halogen exchange is shown below for the synthesis of tritium-labelled mephenytoin. Here the parent molecule is iodinated with N-iodosuccinimide (NIS) in triflic acid, followed by Pd-catalyzed iodine exchange to give the labelled compound Here the parent molecule is iodinated with N-iodosuccinimide (NIS) in triflic acid, followed by Pd-catalyzed iodine exchange to give the labelled compound [3H3](S)-mephenytoin.



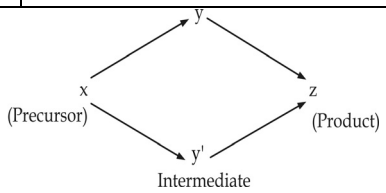
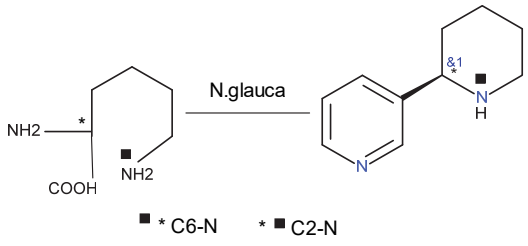
Example of radio labelling

Step-4 Time-to-time determination of radio-activity: After sufficient period of time the metabolite or intermediate or products are isolated to check the radioactivity by suitable detectors. Example: Geiger-Muller Counter, Scintillation Counter. The modern unit of

radioactivity is Becquerel which means onedisintegration per second (dps). The specific detection of radioactive isotopes is straightforward; with liquid scintillation counting (LSC) being the main method by which long-lived radiolabels is quantitatively detected. LSC uses a photomultiplier tube to detect light emissions from the fluor; a fluor is a fluorescent molecule that undergoes excitation by the absorption of radiation and releases light when it relaxes to the ground state. The amount of light emitted by a specified amount of radioactive material can be directly correlated to the amount of radioactivity present.

Step-5 Establishment of precursor-inter-mediate-productrelation: If radioactivity is detected then the precursor-product relationship can be confirmed. Similarly, each step should be elucidated and enzymes catalyzing these steps should be identified.

Various tracer Technique Methods

Tracer technique methods	General Procedure	Example
Precursor Product Sequence	To feed presumed precursor into the plant material, isolate and study radioactivity after pre-decided time to establish precursor product sequence	Restricted synthesis of hyoscine, distinct from hyoscyamine in *Datura stramonium*
 **Fig.1.15** Precursor Product Sequence		
Double and Multiple Labelling	To feed double or multiple labelled precursor to know exact which nitrogen or hydrogen is involved in biosynthesis of product	Doubly labelled lysine used to determine which hydrogen of lysine molecule was involved in formation of piperidine ring of anabasine in *Nicotina glauca*.
Competitive Feeding	To feed two or more precursors to confirm exact precursor-intermediate-product sequence	Biosynthesis of different tropane alkaloids
 **Fig.1.16** Example of competitive feeding		

Contd…

Tracer technique methods	General Procedure	Example
Isotope Incorporation	To feed different isotopes to confirm position of bond cleavage and formation	Glucose – 1- phosphatase cleavage as catalyzed by alkaline phosphatase
Sequential Analysis	To grow plant in atmosphere of $^{14}CO_2$ and then analyze the plant at given time interval to obtain the sequence in which various correlated compound become labelled.	Determination of sequential formation of opium alkaloids

Subjective Questions

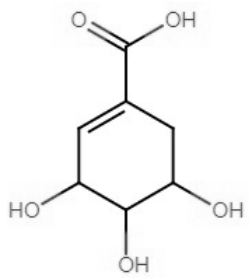
1. Discuss shikimic acid biosynthesis pathways with its significance.
2. Discuss Acetate pathway with its significance.
3. Discuss aromatic amino acid pathway with its significance.
4. How glutamic acid and alanine are biosynthesized?
5. How proline, arginine and orinithine are biosynthesized.
6. How to elucidate biosynthesis pathways by tracer technique?
7. What are different techniques of elucidation of biosynthetic pathways?
8. Which alkaloids are biosynthesized from aromatic amino acids?
9. Why radioactive isotopes are useful in elucidation of biosynthetic pathways?

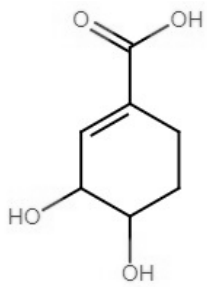
Multiple Choice Questions (MCQs)

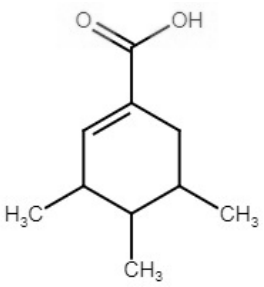
1. Which one of the following technique is used for elucidation of biosynthetic pathway in plants?
   a. X – Ray diffraction
   b. Electron microscope
   c. Tracer technology
   d. None
2. Which labeled isotope is used specifically to understand biosynthesis of alkaloids, proteins and amino acids?
   a. Nitrogen
   b. Sulphur
   c. Carbon
   d. Phosphorus
3. Shikimic acid is synthesized from …….
   a. Hexose phosphate
   b. Pentose phosphate
   c. Heptose phosphate
   d. None

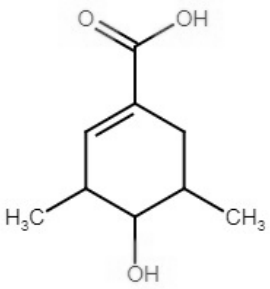
4. Shikimic acid is key intermediate for biosynthesis of ……..
   a. $C_6 – C_3$ units
   b. $C_6 – C_4$ units
   c. $C_3 – C_6$ units
   d. $C_5 – C_3$ units
5. Which one of the following is precursor of shikimic acid?
   a. Chorismic acid
   b. Pyruvic acid
   c. Phosphoenol pyruvic acid
   d. None
6. Which one of the following is immediate precursor of tyrosine in shikimic acid pathway?
   a. P-phenyl pyruvic acid
   b. Prephenic acid
   c. P- hydroxy phenyl pyruvic acid
   d. Chorismic acid
7. Isoprenoids and steroids are biosynthesized by……..
   a. Acetate mevalonate pathway
   b. Both a and b
   c. Shikimic acid pathway
   d. None
8. Fatty acids are synthesized from…….
   a. Acetate Mevalonate pathway
   b. Both a and b
   c. Acetate Mevalonate pathway
   d. None
9. C_5 compound isopentenyl pyrophosphate is derived from mevalonic acid pyrophosphate by
   a. Dehydration and decarboxylation
   b. Dehydration
   c. Decarboxylation and dehydration
   d. Decarboxylation
10. Monoterpenes (C_{10}units) are synthesized from ……..
    a. Farnesyl pyrophosphate
    b. Geranyl pyrophosphate
    c. Isopentenyl pyrophosphate
    d. None
11. C_{15} unit isoprenoid compounds are synthesized from……..
    a. Farnesyl pyrophosphate
    b. Geranyl pyrophosphate
    c. Isopentenyl pyrophosphate
    d. None
12. Squalene is precursor of ……
    a. Steroids
    b. Diterpenes
    c. Triterpenes
    d. Both a and b
13. Choose the correct sequence involved in cholesterol synthesis from the following
    a. Acetate ,Malonte, isopentenyl pyrophosphate, Squalene pathway
    b. Acetate, Mevalonate , isopentenyl pyrophosphate, Squalene pathway
    c. Acetate , Mevalonate, pentenyl pyrophosphate , Squalene pathway
    d. Acetate, Mevalonate , isopentenyl phosphate , Squalene pathway

14. Choose the correct sequence
    a. Shikimic acid Chorismic acid Anthranilic acid Tryptophan
    b. Shikimic acid Prephenic acid Anthranilic acid Tryptophan
    c. Shikimic acid Chorismic acid Anthranilic acid Tryptophan
    d. Shikimic acid Chorismic acid Phenyl pyruvic acid Tryptophan
15. Choose the correct sequence
    a. Shikimic acid Prephenic acid Phenyl pyruvic acid Phenylalanine
    b. Shikimic acid Chorismic acid Phenyl pyruvic acid Phenylalanine
    c. Shikimic acid Chorismic acid Phenyl pyruvic acid Tyrosine
    d. Shikimic acid Prephenic acid Phenyl pyruvic acid Tyrosine
16. The radioisotopes used for tracing the biosynthetic pathways are…….
    a. ^{14}C, ^{3}H, ^{36}S, ^{32}P
    b. ^{15}C, ^{1}H, ^{34}S, ^{34}P
    c. ^{13}C, ^{2}H, ^{34}S, ^{30}P
    d. ^{11}C, ^{2}H, ^{33}S, ^{31}P
17. Which …… amino acid is derived from shikimic acid pathway?
    a. Tyrosine
    b. Isoleucine
    c. Valine
    d. Phenylanine
18. Malonyl CoA is precursor of ……..
    a. Lipids
    b. flavonoids
    c. Steroids
    d. Glycosides
19. IUPAC name of mevalonic acid is……..
    a. 2,3,5 – trihydroxy – 3- methylavaleric acid
    b. 5-hydroxy-3-methylavaleric acid
    c. 3-hydroxy -3-methylavaleric acid
    d. 3, 5 – dihydroxy -3-methylavaleric acid
20. Which one of the following is structure of shikimic acid?

a. 

c. 

b. 

d. 

Answer Key

1. c	2. a	3. c	4. a	5. b	6. b	7. a	8. a	9. b	10. C
11. a	12. d	13. a	14. b	15. a	16. a	17. b	18. a	19. d	20. a