UNIT-I

Metabolic pathways in higher plants and their determination

a) Brief study of basic metabolic pathways and formation of different secondary metabolites Through these pathways- Shikimic acid pathway, Acetate pathways and Amino acid pathway.

b) Study of utilization of radioactive isotopes in the investigation of Biogenetic studies.

Plant secondary metabolism

Secondary metabolism produces a large number of specialized compounds (estimated 200,000) that do not aid in the growth and development of plants but are required for the plant to survive in its environment. Secondary metabolism is connected to primary metabolism by using building blocks and biosynthetic enzymes derived from primary metabolism. Primary metabolism governs all basic physiological processes that allow a plant to grow and set seeds, by translating the genetic code into proteins, carbohydrates, and amino acids. Specialized compounds from secondary metabolism are essential for communicating with other organisms in mutualistic (e.g. attraction of beneficial organisms such as pollinators) or antagonistic interactions (e.g. deterrent against herbivores and pathogens). In any case, a good balance between products of primary and secondary metabolism is best for a plant's optimal growth and development as well as for its effective coping with often changing environmental conditions. Well known specialized compounds include alkaloids, polyphenols including flavonoids, and terpenoids. Humans use quite a lot of these compounds, or the plants from which they originate, for culinary, medicinal and nutraceutical purposes.

Primary *vs* Secondary Plant Metabolism

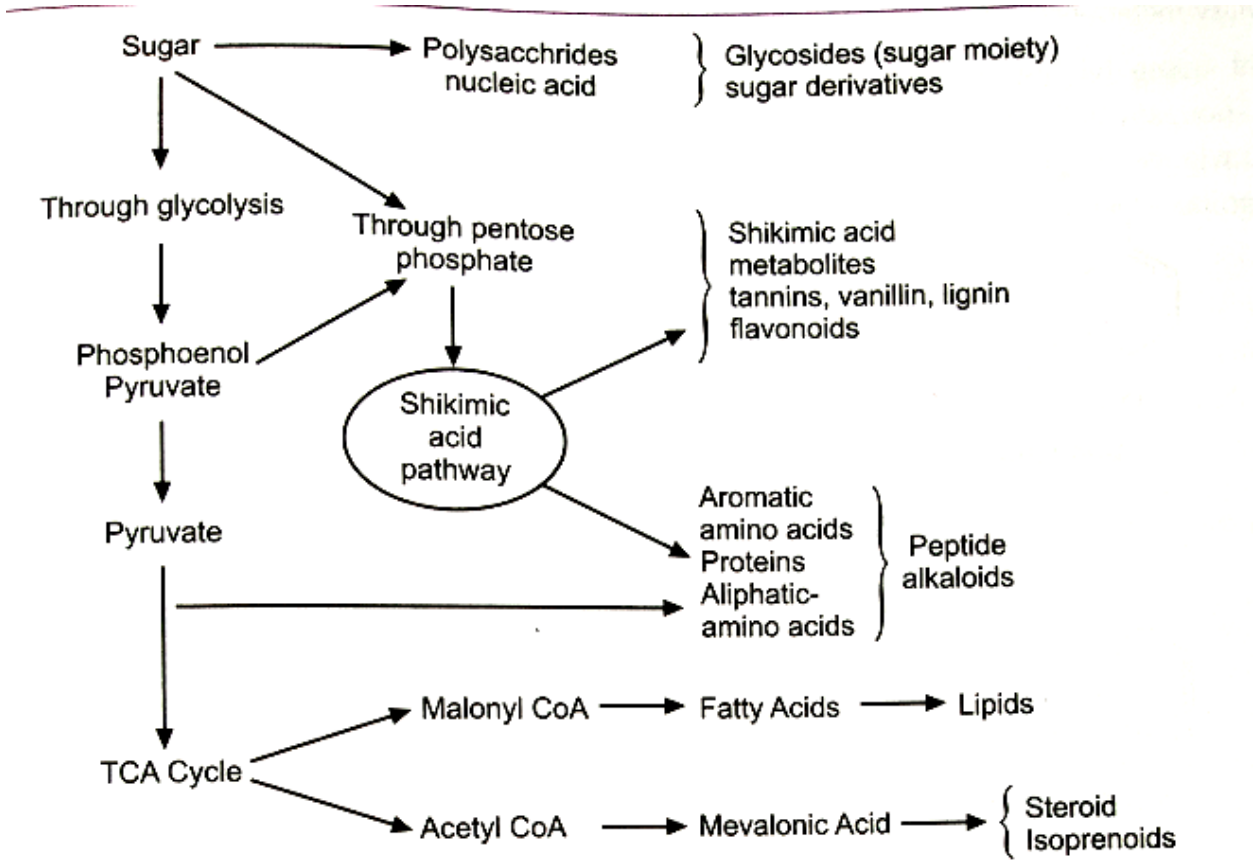
Primary metabolism in a plant comprises all metabolic pathways that are essential to the plant's survival. Primary metabolites are compounds that are directly involved in the growth and development of a plant whereas secondary metabolites are compounds produced in other metabolic pathways that, although important, are not essential to the functioning of the plant. However, secondary plant metabolites are useful in the long term, often for defense purposes, and give plants characteristics such as color. Secondary plant metabolites are also used in signalling and regulation of primary metabolic pathways. Plant hormones, which are secondary metabolites, are often used to regulate the metabolic activity within cells and oversee the overall development of the plant. Secondary plant metabolites help the plant maintain an intricate balance with the environment, often adapting to match the environmental needs. Plant metabolites that color the plant are a good example of this, as the coloring of a plant can attract pollinators and also defend against attack by animals.

Types of Secondary Metabolites in plants

There is no fixed, commonly agreed upon system for classifying secondary metabolites. Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups:

- Flavonoids and allied phenolic and polyphenolic compounds,
- Terpenoids and
- Nitrogen-containing alkaloids and sulphur-containing compounds.

Primary and secondary metabolites derived from carbon metabolism in plants



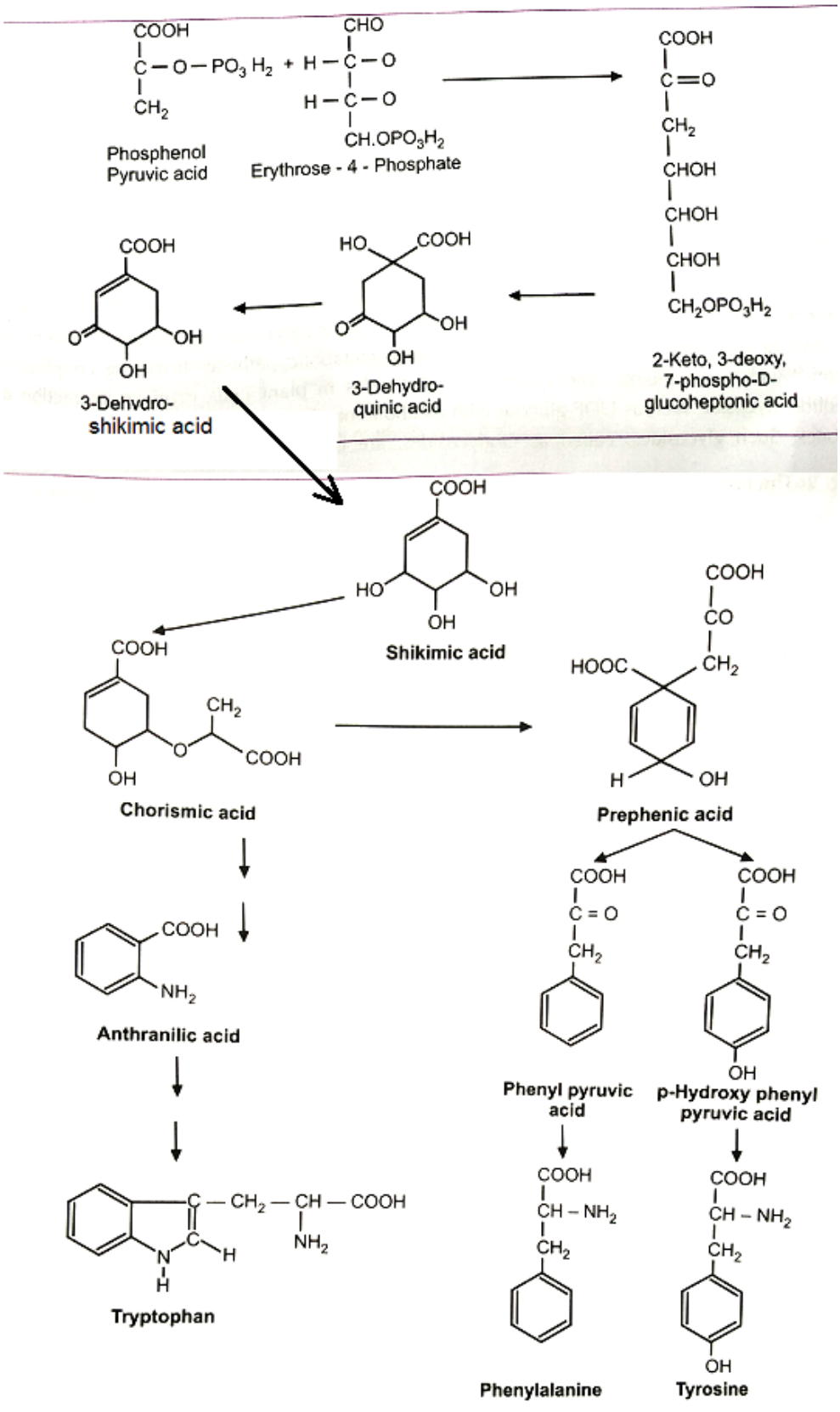
Shikimic Acid Pathway

The shikimate pathway (shikimic acid pathway) is a seven-step metabolic pathway used by bacteria, fungi, algae, some protozoans, and plants for the biosynthesis of folates and aromatic amino acids (phenylalanine, tyrosine, and tryptophan). This pathway is not found in animals (including humans), who must instead obtain these essential amino acids from their diet. This can be through either the direct consumption of plants or microorganisms, or their indirect consumption via the consumption of other animals; therefore, phenylalanine and tryptophan represent essential amino acids that must be obtained from the animal's diet. Animals can

synthesize tyrosine from phenylalanine, and therefore is not an essential amino acid except for individuals unable to hydroxylate phenylalanine to tyrosine).

Shikimic acid

Commonly known as its anionic form **shikimate**, is a cyclohexene, a cyclitol and a cyclohexanecarboxylic acid. Shikimic acid is a key intermediate from carbohydrate for the biosynthesis of $C_6 - C_3$ units (Phenylpropane derivatives). Besides serving as precursor for the biosynthesis of amino acids, it is also an intermediate in production of tannins, flavones, coumarins and vanillin.



Shikimic acid pathway

Role of Shikimic Acid Pathway:

- **Starting material in the biosynthesis of some phenolics**

  Phenyl alanine and **tyrosine** are the precursors used in the biosynthesis of phenylpropanoids. The phenylpropanoids are then used to produce the *flavonoids, coumarins, tannins and lignin.*

- **Gallic acid biosynthesis**

  Gallic acid is formed from *3-dehydroshikimate* by the action of the *enzyme shikimate dehydrogenase* to produce *3,5-didehydroshikimate*. The latter compound spontaneously rearranges to gallic acid.

- Shikimic acid is a precursor for indole, indole derivatives and aromatic amino acid tryptophan and tryptophan derivatives such as the psychedelic compound dimethyltryptamine. many alkaloids and other aromatic metabolites.

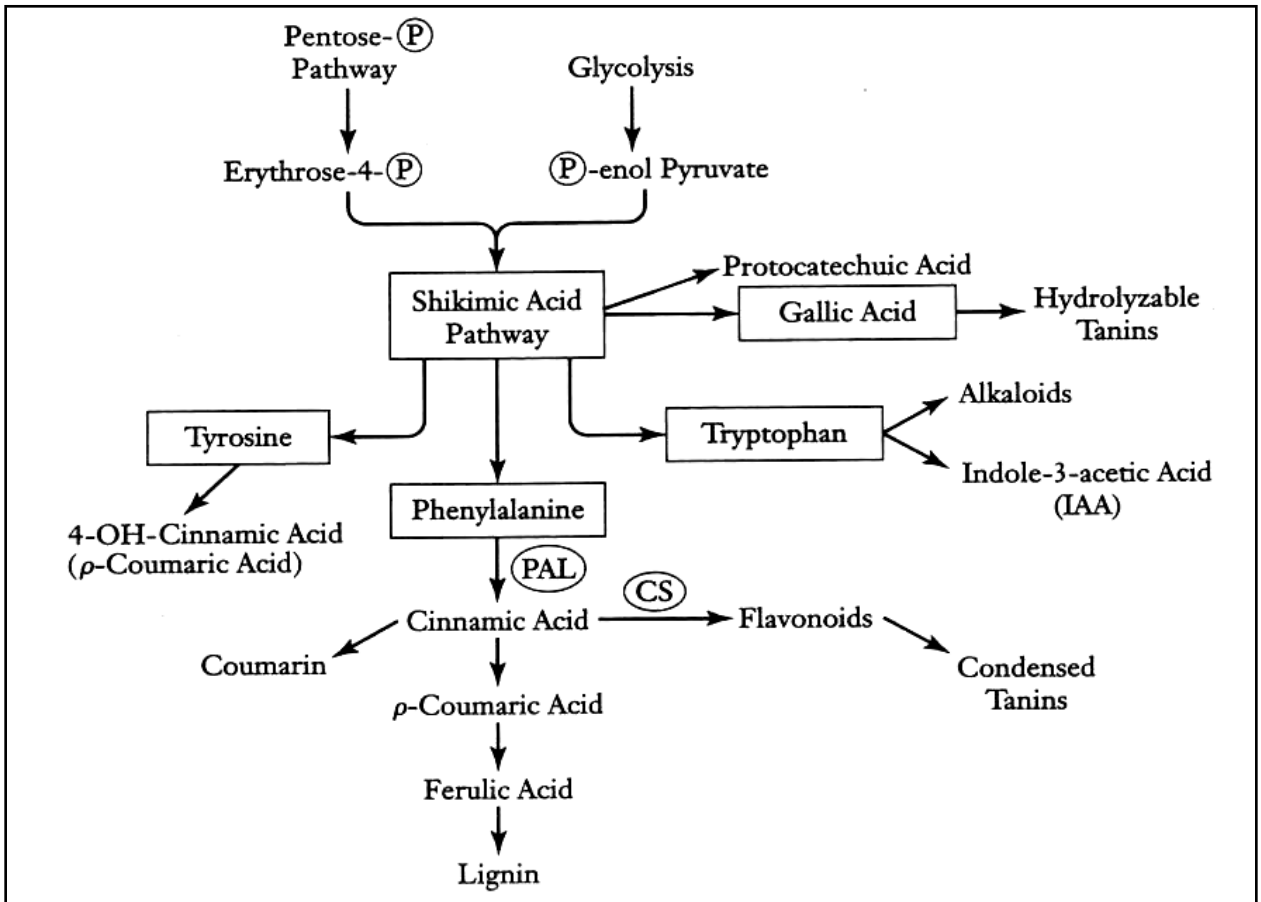


FIGURE 14.14 The central role of the shikimic acid pathway in the synthesis of various primary and secondary metabolites. PAL = phenylalanine ammonia lyase. CS = chalcone synthase.

Mevalonate pathway

The mevalonate pathway, also known as the isoprenoid pathway or HMG-CoA reductase pathway is an essential metabolic pathway present in eukaryotes, archaea, and some bacteria. The pathway produces two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are used to make isoprenoids, a diverse class of over 30,000 biomolecules such as cholesterol, vitamin K, coenzyme Q10, and all steroid hormones.

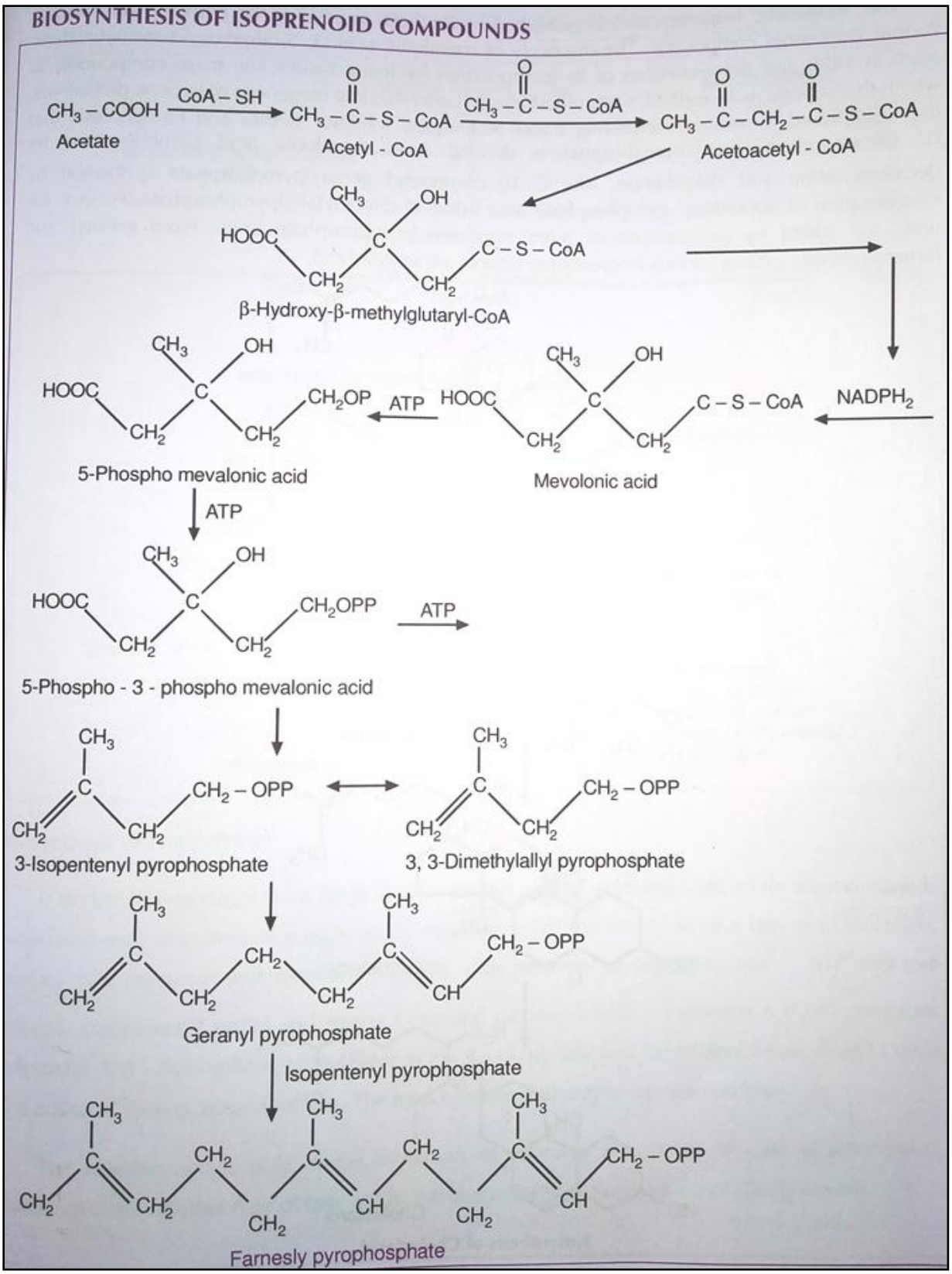
Upper mevalonate pathway

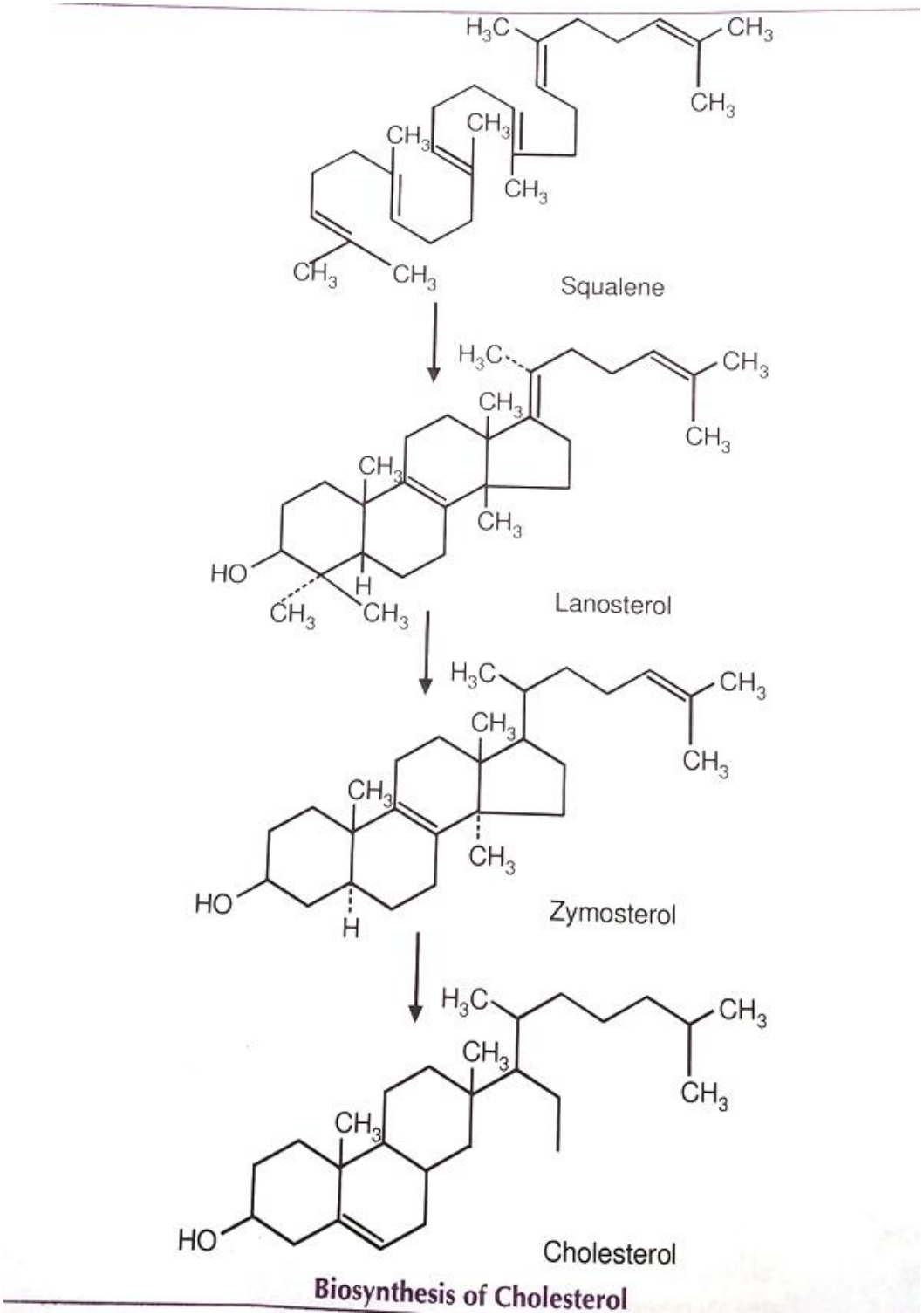
The mevalonate pathway of eukaryotes, archaea, and eubacteria all begin the same way. The sole carbon feed stock of the pathway is acetyl-CoA. The first step condenses two acetyl-CoA molecules to yield acetoacetyl-CoA. This is followed by a second condensation to form HMG-CoA (3-hydroxy-3- methyl-glutaryl-CoA). Reduction of HMG-CoA yields (R)-mevalonate. These first 3 enzymatic steps are called the upper mevalonate pathway.

Lower mevalonate pathway

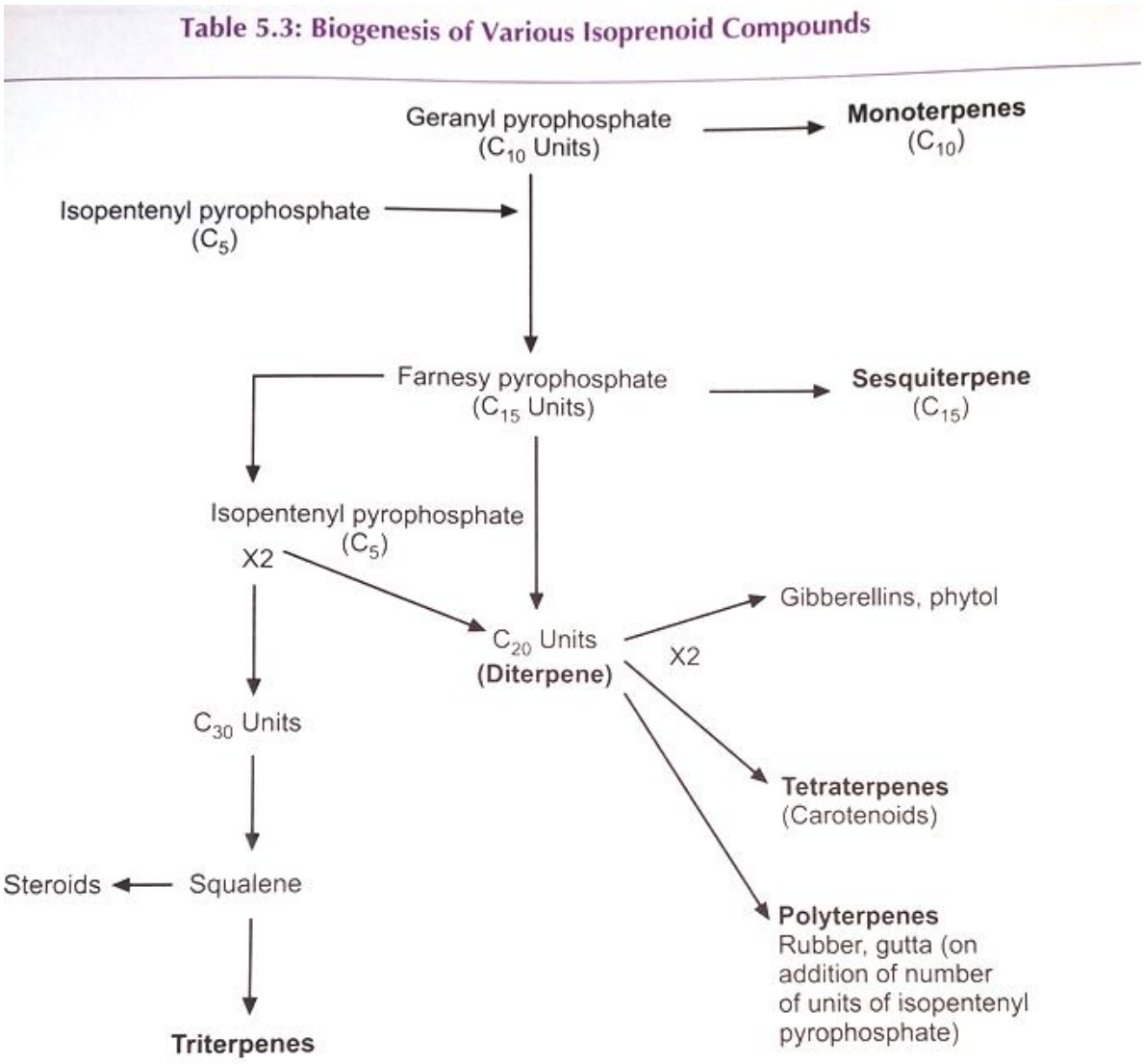
The lower mevalonate pathway which converts (R)-mevalonate into IPP and DMAPP has 3 variants. In eukaryotes, mevalonate is phosphorylated twice in the 5-OH position, then decarboxylated to yield IPP. In some archaea such as *Haloferax volcanii*, mevalonate is phosphorylated once in the 5-OH position, decarboxylated to yield isopentenyl phosphate (IP), and finally phosphorylated again to yield IPP (Archaeal Mevalonate Pathway I). A third mevalonate pathway variant found in Thermoplasma acidophilum, phosphorylates mevalonate at the 3-OH position followed by phosphorylation at the 5-OH position. The resulting metabolite, mevalonate-3,5-bisphosphate, is decarboxylated to IP, and finally phosphorylated to yield IPP (Archaeal Mevalonate Pathway II).

BIOSYNTHESIS OF ISOPRENOID COMPOUNDS





Biosynthesis of Cholesterol



Amino acid pathway

Amino acids

Amino acids are organic compounds containing amine (-NH2) and carboxyl (-COOH) functional groups, along with a side chain (R) group specific to each amino acid. Many amino acids contain only carbon, hydrogen, oxygen and nitrogen, but other atoms may be present (e.g. sulphur in cystine, and iodine in thyroxin). As already mentioned, more than one amino group may be present (e.g. Lysine, diaminocaproic acid) and more than one carboxylic acid group (e.g. aspartic or amino succinic acid). Some amino acids are aromatics such as phenylalanine, or heterocyclic

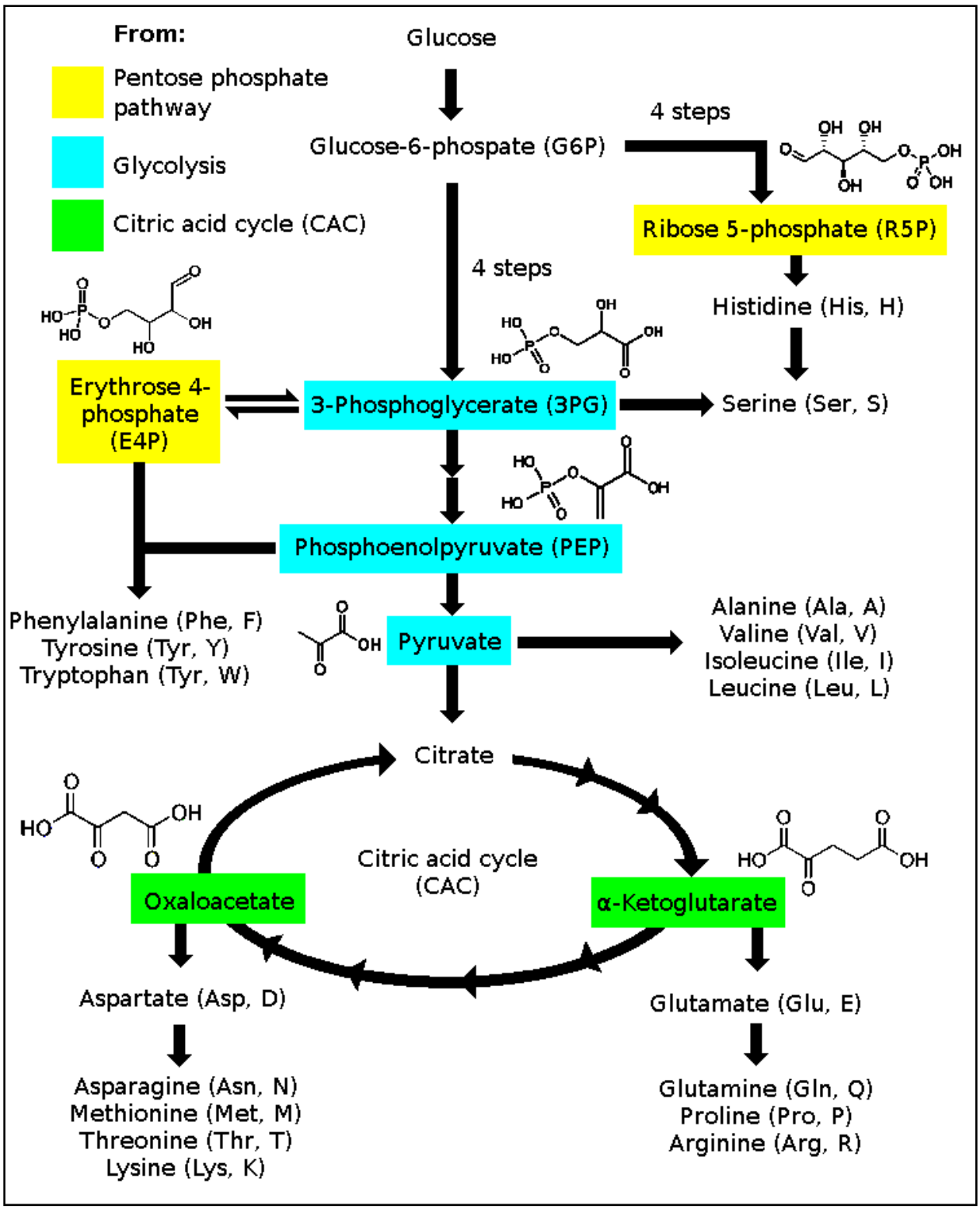
such as proline (pyrolidine nucleus), tryptophan (indole nucleus) and histidine (imidazole nucleus).

Essential amino acids	Non-essential amino acids
Arginine	Alanine
HIstidine	Asparagine
Isoleucine	Aspartate
Lysine	Cysteine
Methionine	Glutamate
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
Leucine	Tyrosine

Amino acid synthesis is the set of biochemical processes (metabolic pathways) by which the amino acids are produced. The substrates for these processes are various compounds in the organism's diet or growth media. Not all organisms are able to synthesize all amino acids. For example, humans can only synthesize 11 of the 20 standard amino acids (a.k.a. non-essential amino acid), and in time of accelerated growth, histidine can be considered an essential amino acid.

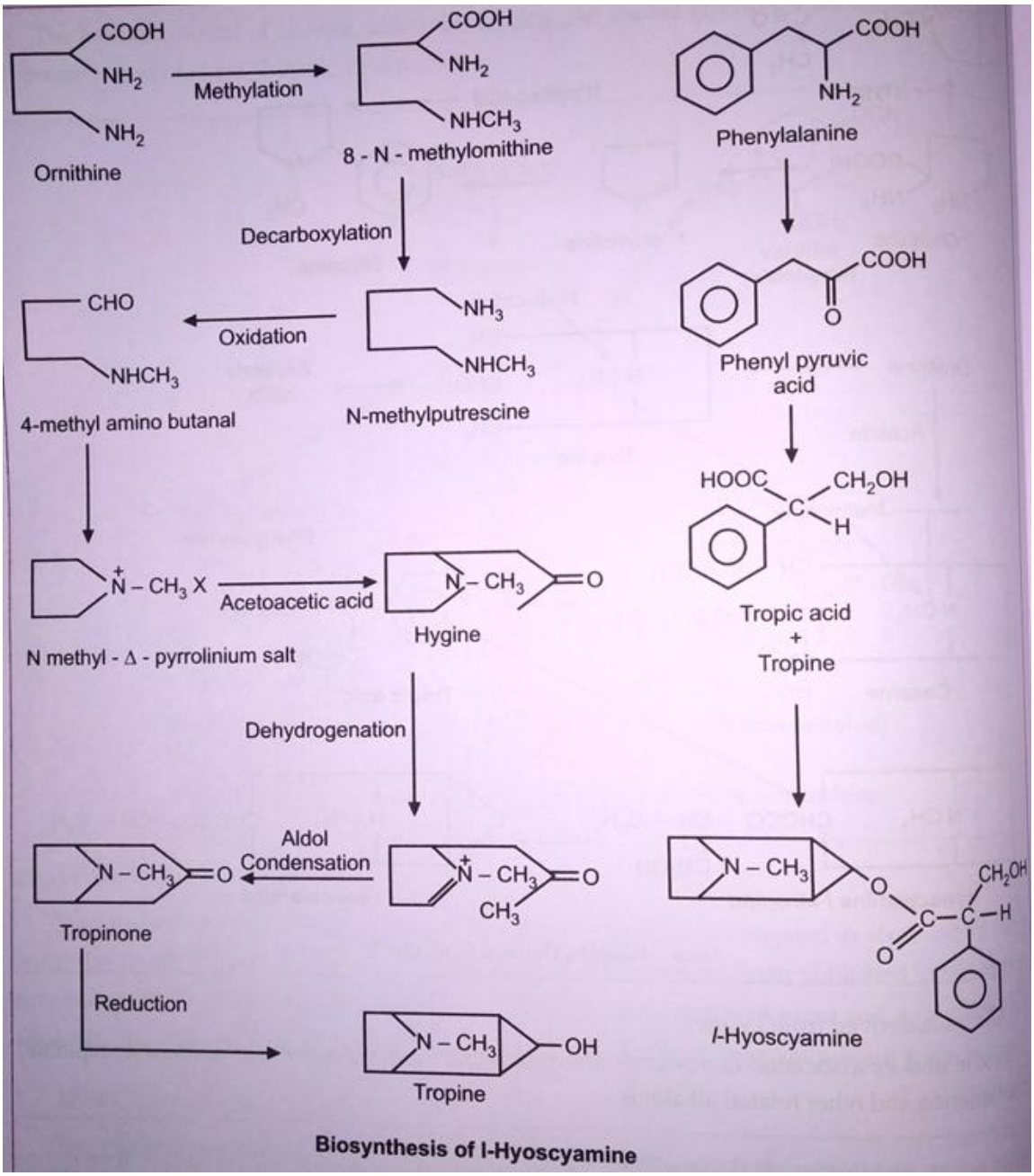
Of the basic set of twenty amino acids (not counting selenocysteine), humans cannot synthesize eight. In addition, the amino acids arginine, cysteine, glycine, glutamine, histidine, proline, serine, and tyrosine are considered conditionally essential, meaning they are not normally required in the diet but must be supplied exogenously to specific populations that do not synthesize it in adequate amounts. For example, enough arginine is synthesized by the urea cycle to meet the needs of an adult but perhaps not those of a growing child. Amino acids that must be obtained from the diet are called essential amino acids. Nonessential amino acids are produced in the body. The pathways for the synthesis of nonessential amino acids are quite simple. Glutamate dehydrogenase catalyzes the reductive amination of α-ketoglutarate to glutamate. A transamination reaction takes place in the synthesis of most amino acids. At this step, the

chirality of the amino acid is established. Alanine and aspartate are synthesized by the transamination of pyruvate and oxaloacetate, respectively. Glutamine is synthesized from NH_4^+ and glutamate, and asparagine is synthesized similarly. Proline and arginine are derived from glutamate. Serine, formed from 3-phosphoglycerate, is the precursor of glycine and cysteine. Tyrosine is synthesized by the hydroxylation of phenylalanine, an essential amino acid. The pathways for the biosynthesis of essential amino acids are much more complex than those for the nonessential ones.



Amino acid pathway

Alkaloids derived from ornithine



Radioactive isotopes in the investigation of Biogenetic studies

Radioactive isotope, also called radioisotope, radionuclide, or radioactive nuclide, any of several species of the same chemical element with different masses whose nuclei are unstable and dissipate excess energy by spontaneously emitting radiation in the form of alpha, beta, and

gamma rays. Radioactive isotopes have many useful applications. In medicine, for example, cobalt-60 is extensively employed as a radiation source to arrest the development of cancer. Other radioactive isotopes are used as tracers for diagnostic purposes as well as in research on metabolic processes. When a radioactive isotope is added in small amounts to comparatively large quantities of the stable element, it behaves exactly the same as the ordinary isotope chemically; it can, however, be traced with a Geiger counter or other detection device. Iodine-131 has proved effective in treating hyperthyroidism. Another medically important radioactive isotope is carbon-14, which is used in a breath test to detect the ulcer-causing bacteria *Heliobacter pylori*.

TRACER TECHNIQUES

It can be defined as technique which utilizes a labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time. In this technique different isotope, mainly the radioactive isotopes which are incorporated into presumed precursor of plant metabolites and are used as marker in biogenic experiments.

What is labelled compounds

The labelled compound can be prepared by use of two types of isotopes.

1. Radioactive isotopes 2. Stable isotopes

Radioactive isotopes: - [e.g. ^{1}H, ^{14}C, ^{24}Na, ^{42}K, ^{35}S, ^{35}P, ^{131}I decay with emission of radiation]

- For biological investigation – carbon & hydrogen.
- For metabolic studies – S, P, and alkali and alkaline earth metals are used.
- For studies on protein, alkaloids, and amino acid – labelled nitrogen atom give more specific information.
- ^{3}H compound is commercially available.

- **Stable isotopes: -** [e.g. ^{2}H, ^{13}C, ^{15}N, ^{18}O]
  - Used for labeling compounds as possible intermediates in biosynthetic pathways.
  - Usual method of detection are: – MASS spectroscopy [^{15}N, ^{18}O
  - NMR spectroscopy [^{2}H, ^{13}C]

METHODS IN TRACER TECHNIQUE

1. Precursor Product Sequence

In this technique, the presumed precursor of the constituent under investigation on a labelled form is fed into the plant and after a suitable time the constituent is isolated, purified and radioactivity is determined.

Disadvantage: - The radioactivity of isolated compound alone is not usually sufficient evidence that the particular compound fed is direct precursor, because substance may enter the general metabolic pathway and from there may become randomly distributed through a whole range of product.

Application: -

- Stopping of hordenine production in barley seedling after 15 – 20 days of germination.
- Restricted synthesis of hyoscine, distinct from hyoscyamine in *Datura stramonium*.
- This method is applied to the biogenesis of morphine & ergot alkaloids

2. Double and Multiple Labelling

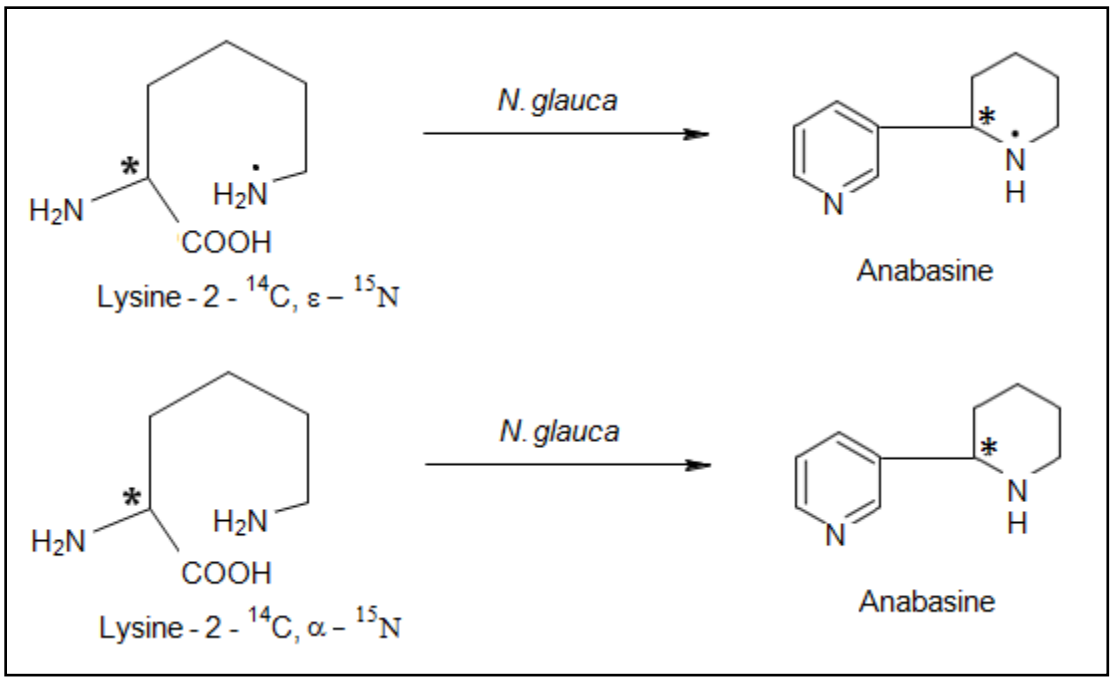
This method give the evidence for nature of biochemical incorporation of precursor arises double & triple labelling. In this method specifically labelled precursor and their subsequent degradation of recover product are more employed.

Application

This method is extensively applied to study the biogenesis of plant secondary metabolites.

Used for study of morphine alkaloid.

E.g. Leete, use doubly labeled lysine used to determine which hydrogen of lysine molecule was involved in formation of piperidine ring of anabasine in *Nicotina glauca*.

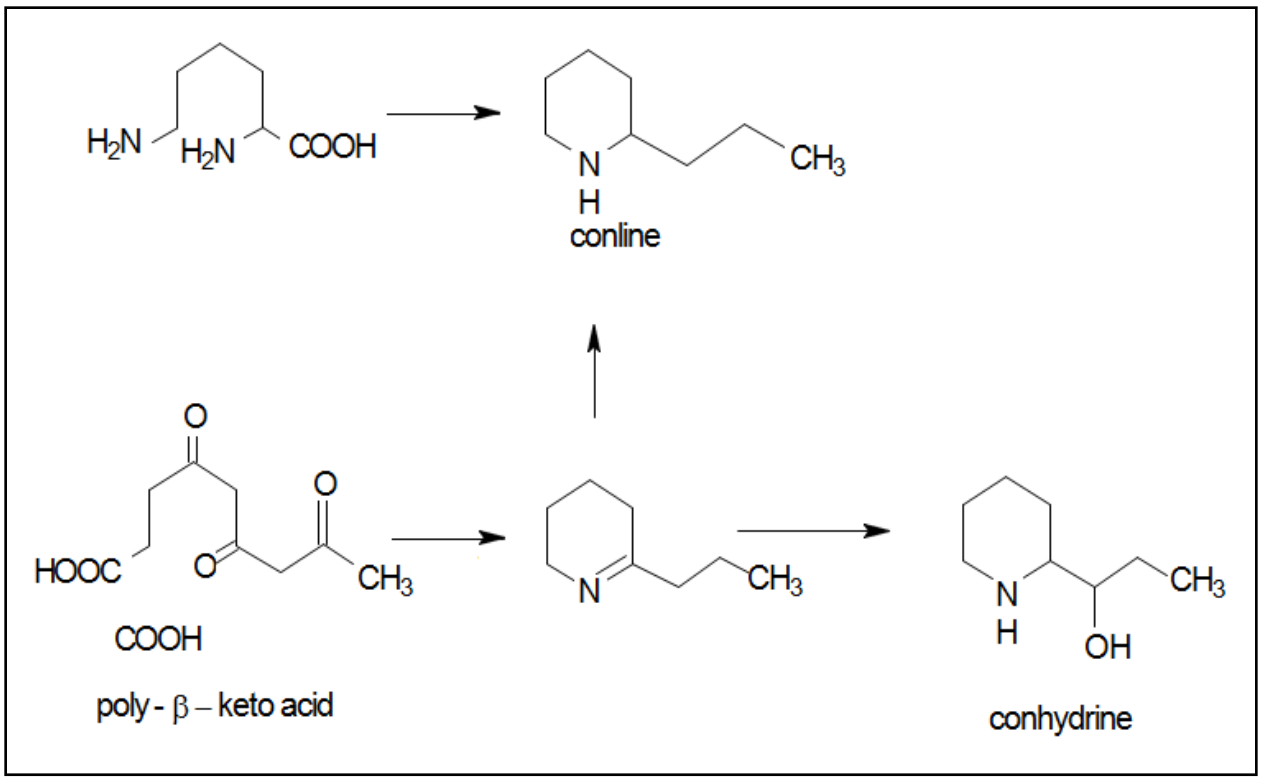


3. Competitive Feeding

If incorporation is obtained it is necessary to consider whether this in fact, the normal route of synthesis in plant not the subsidiary pathway. Competitive feeding can distinguish whether B & B' is normal intermediate in the formation of C from A.

Applications

- This method is used for elucidation of biogenesis of propane alkaloids.
- Biosynthesis of hemlock alkaloids (conline, conhydrine etc) e.g. biosynthesis of alkaloids of *Conium maculactum* (hemlock) using ^{14}C labelled compounds.



4. Isotope Incorporation

This method provides information about the position of bond cleavage & their formation during reaction.

E.g. Glucose – 1- phosphatase cleavage as catalyzed by alkaline phosphatase this reaction occur with cleavage of either C – O bond or P – O bond.

5. Sequential Analysis: - The principle of this method of investigation is 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.

Application

- $^{14}CO_2$ & sequential analysis has been very successfully used in elucidation of carbon in photosynthesis.
- Determination of sequential formation of opium hemlock and tobacco alkaloids.
- Exposure as less as 5 min. $^{14}CO_2$, is used in detecting biosynthetic sequence as – Piperitone ⟶ (-) Menthone ⟶ (-) Menthol in *Mentha piperita*.

APPLICATION OF TRACER TECHNIQUE

- Study of squalene cyclization by use of ^{14}C, ^{3}H labelled mevalonic acid.
- Interrelationship among 4 – methyl sterols and 4, 4 dimethyl sterols, by use of ^{14}C acetate.
- Terpenoid biosynthesis by chloroplast isolated in organic solvent, by use of 2- ^{14}C mevalonate.
- Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin.
- Origin of carbon & nitrogen atoms of purine ring system by use of ^{14}C or ^{15}N labelled precursor.
- Study of formation of scopoletin by use of labelled phenylalanine.
- By use of ^{45}Ca as tracer, - found that the uptake of calcium by plants from the soil. (CaO & CaCO2).
- By adding ammonium phosphate labelled with ^{32}P of known specific activity the uptake of phosphorus is followed by measuring the radioactivity as label reaches first in lower part of plant, than the upper part *i.e.* branches, leaves etc.

YouTube Video links

1. https://youtu.be/v1vqV7YHKWg
2. https://youtu.be/AresORA0bwQ
3. https://youtu.be/CWhTA0e7dKw
4. https://youtu.be/ZT4DzfIRMeQ