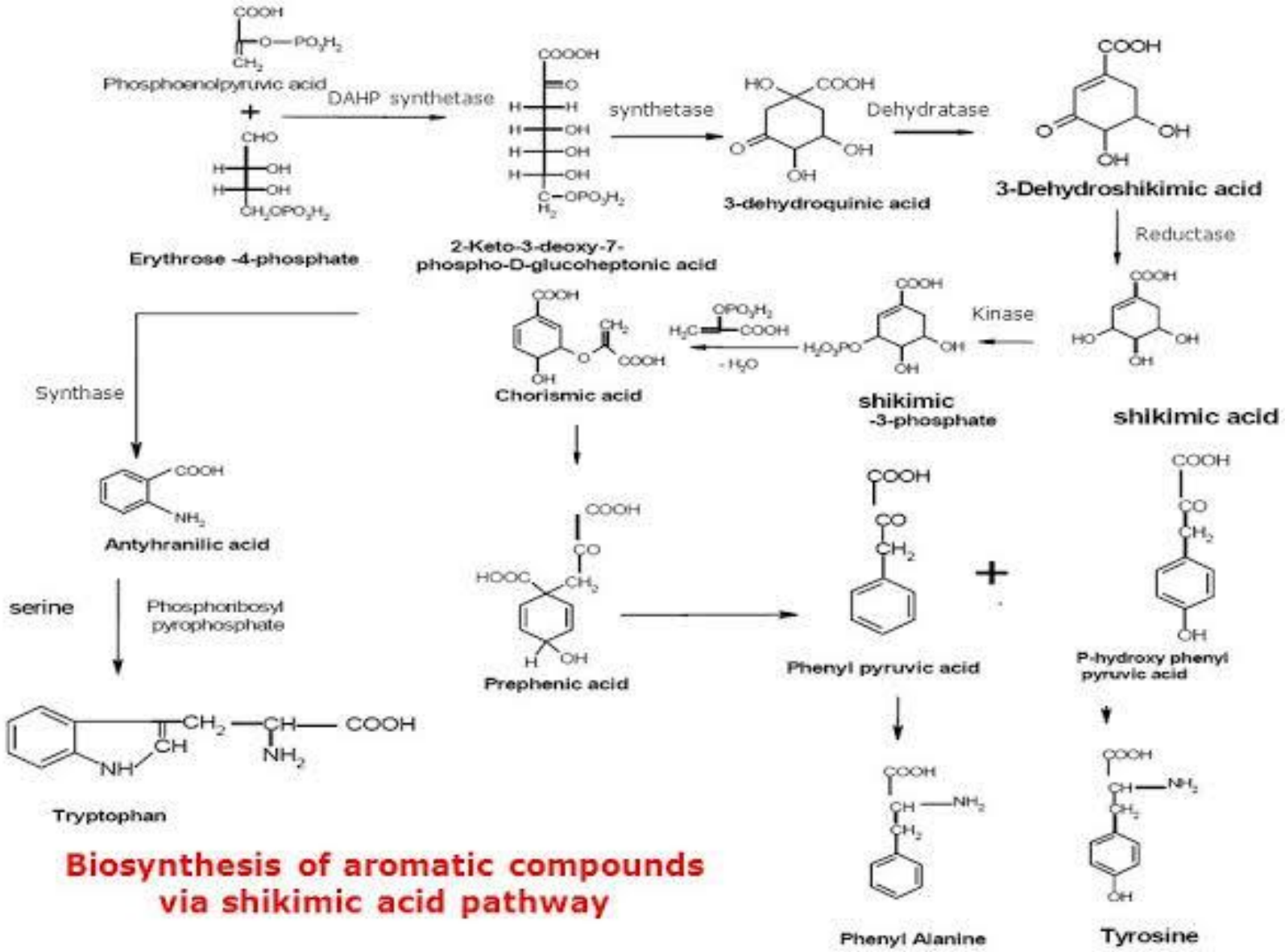
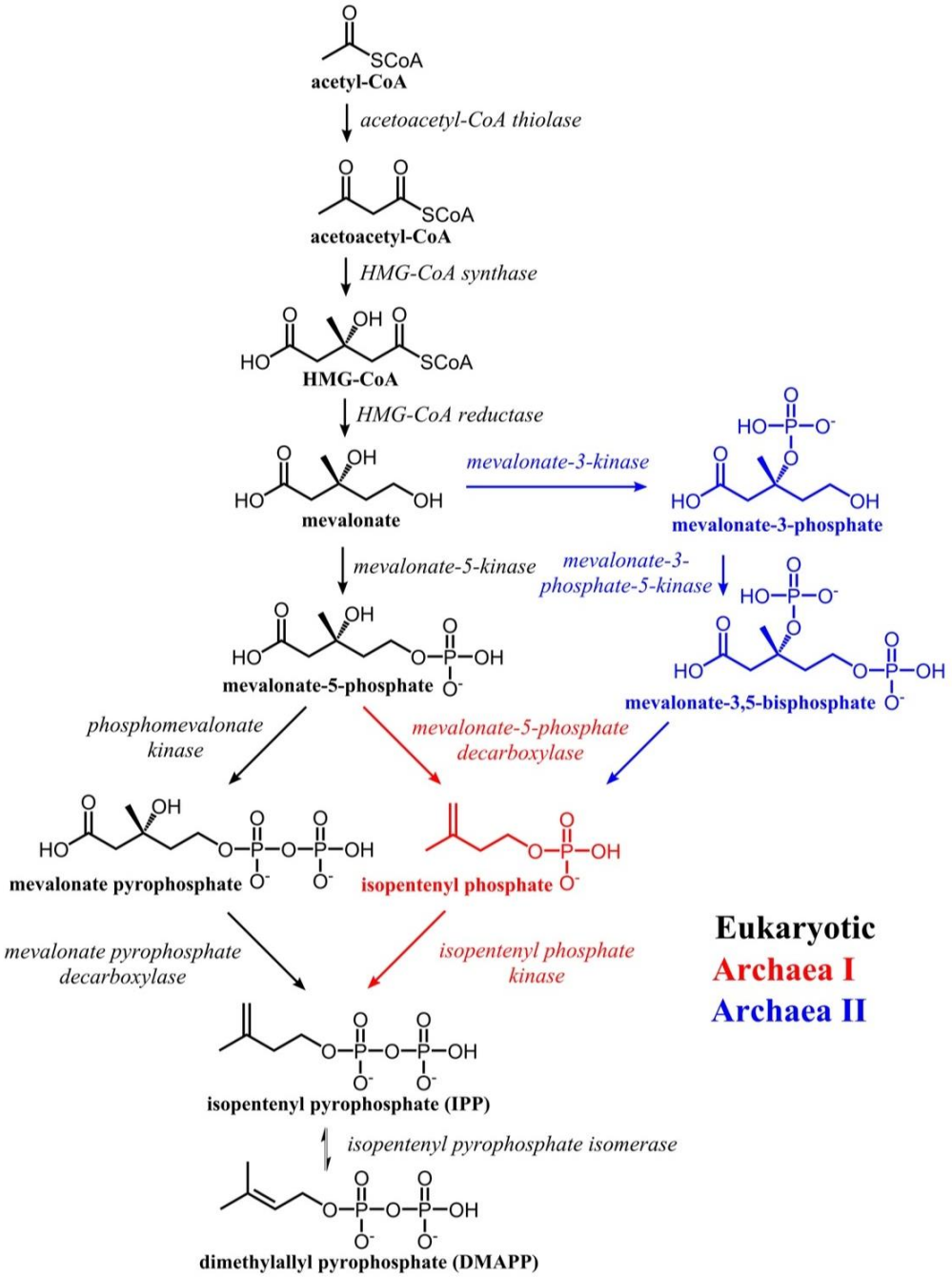
Shikimic acid pathway-

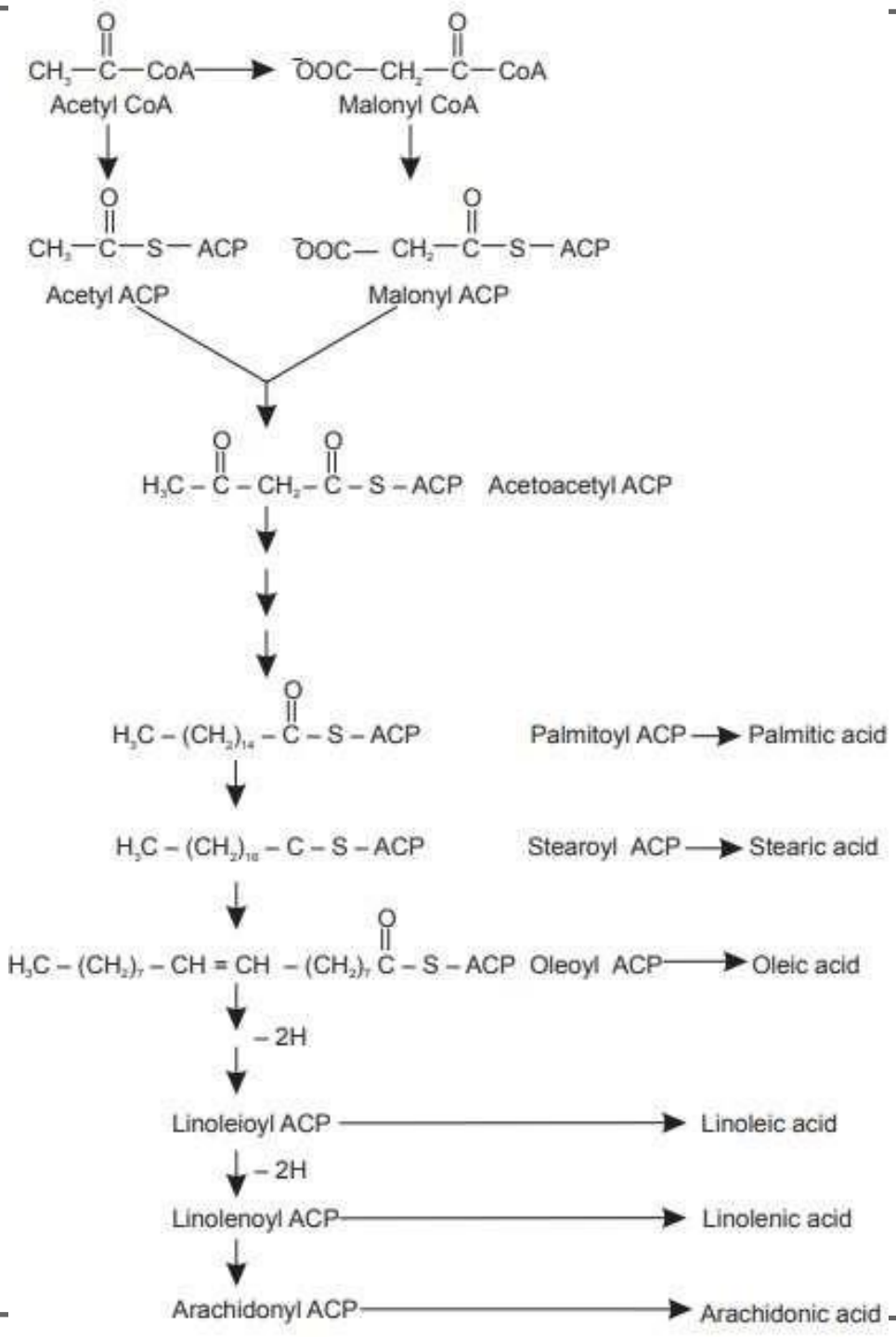


Biosynthesis of aromatic compounds via shikimic acid pathway

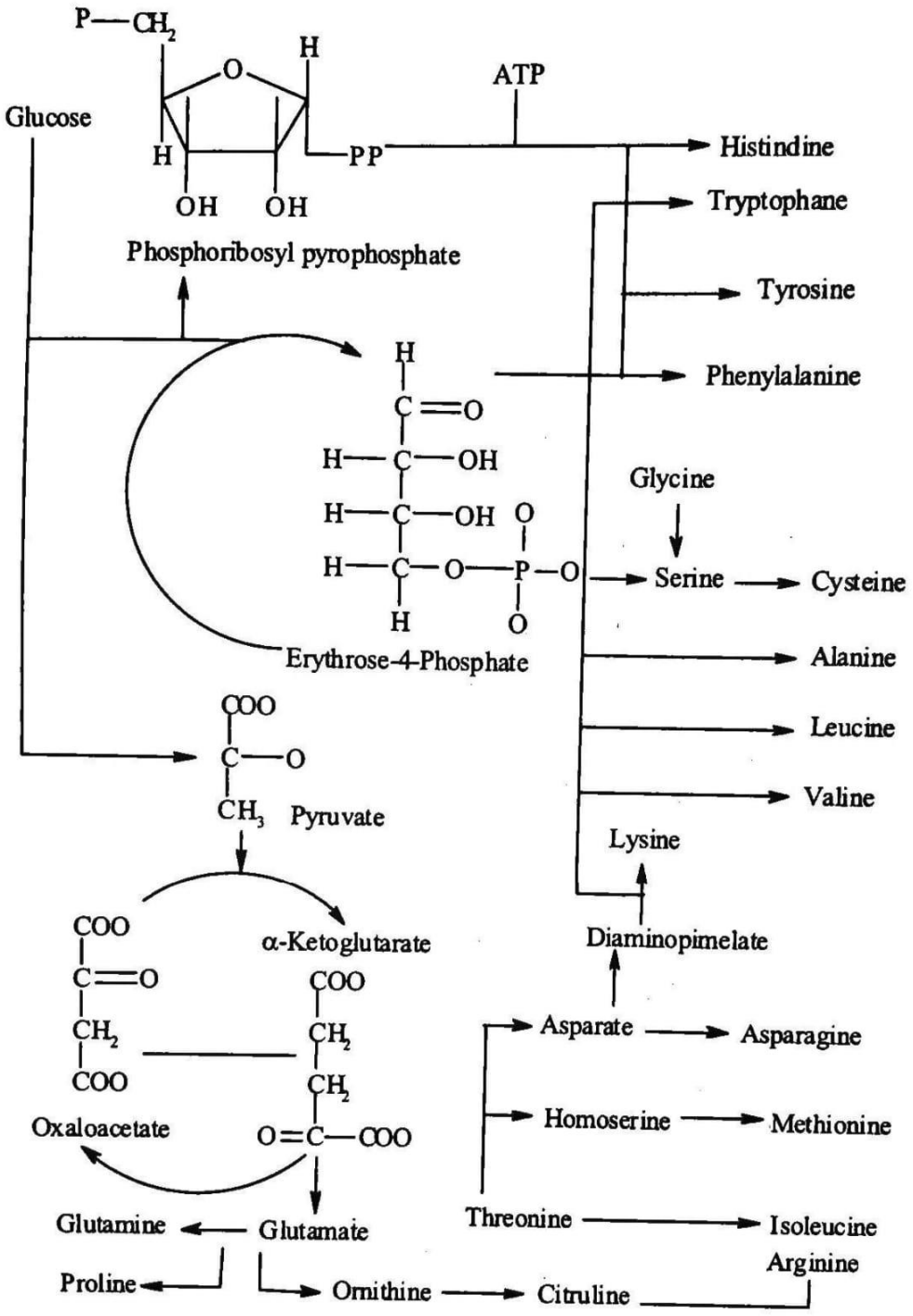
Acetate mevolanate pathway-



Acetate melonate pathway-



Amino acid pathway



Utilisation Of Radioactive Isotopes In The Investigation Of Biogenetic Studies

These are techniques used for the investigation of biosynthetic pathway
1. Tracer technique
2. Use of isolated organ and tissues
3.Grafting method
4. Use of Mutant strains

1. Tracer technique:

Tracer techniques utilizes radioactive isotope labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time

The following two types of isotopes are used for labelling:
1) Radioactive Isotopes: 1H, 14C, 24Na, 42K, 35S, 35P, and 131I are the examples of such isotopes.

2) Stable Isotopes: 2H, 13C, 15N, and 18O are the examples of such isotopes.

Significance of Tracer Technique
The tracer technique has the following significances:
1) It is highly sensitive.
2) It is used in the living system

Steps Involved in Tracer Technique

Following four steps are involved in the tracer technique:

Step 1: Preparation of Labelled Compound

The labelled compound is produced by allowing them to grow in atmosphere of $14CO_2$. All compounds containing carbon get 14C labelled.

Step 2: Introduction of Labelled Compound into Biological System

1) Root Feeding: This method is preferred in plants in which the roots are the sites of biosynthesis, e.g., tobacco. In this method, the plants are cultivated hydroponically to prevent them from microbial contamination.

2) Stem Feeding: This method does not require root for biosynthesis. This is because the substrate can be administered through the cut ends of stem immersed in a solution. This method of labelling is not suited for plants containing latex.

Step 3: Separation and Detection Techniques

1) Soft and Fresh Tissue: Infusion and maceration.
2) Hard Tissue: Decoction and hot percolation.
3) Unorganised Drug: Maceration with adjustment.

Step 4: Methods for Tracer Technique

1) Precursor Product Sequence:

In this method, the presumed precursor of the plant constituent to be investigated on a labelled fo rm is introduced into the plant. After some time , the constituent is isolated, purified and its radioactivity is measured.

Examples

i) In Datura stramonium, the restricted synthesis of hyoscine is different from hyoscyamine.

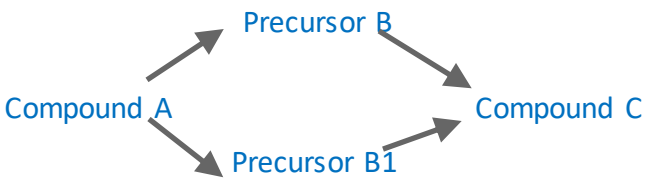
2)competitive Feeding:

If incorporation is obtained it is still necessary to consider whether this is in fact the normal route of synthesis in the plant and not a subsidiary pathway invoked as a result of the atypical availability to the plant of the administered compound

Competitive feeling experiments can be of value in determining which of two possible intermediate is normally used by the plant

By this method one can accurately determine the actual per cursor involved in the biosynthesis of a particular metabolites

To percursor are then introduced into two separate group of plant if the radioactivity is observed in the group receiving precursor B and not in B1 receiving group then the biosynthetic pathway for the particular metabolites follows order A → B → C but not A → B1 → C



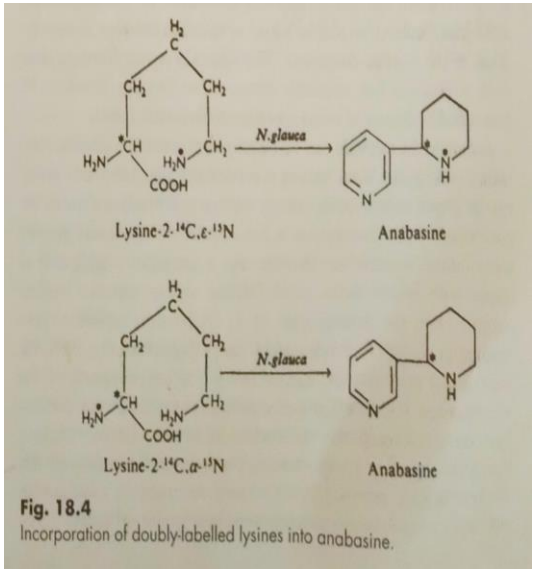


Fig. 18.4
Incorporation of doubly-labelled lysines into anabasine.

3)Sequential Analysis:

In this method, the plant is allowed to grow in atmosphere of $14CO_2$ and then the plant growth at given time interval is analysed to obtain the sequence in which various correlated compound s become labelled.

Examples

i) In photosynthesis,

$14CO_2$ sequential analysis is used in carbon elucidation.

Applications:

$14CO_2$ and sequential analysis has been very successfully used in elucidation of carbon in photosynthesis.

Determination of sequential formation of opium and tobacco alkaloids.

4)Double and Multiple Labelling

This method provides evidences for the nature of biochemical incorporation of precursor that give rise to double and triple labelling .

5)Isotope incorporation:

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

Example: the cleavage of glucose -1- phosphotase is catalysed by alkaline phosphatase.

Reaction occurs with cleavage of either C-O bond/P-O bond. If the reaction is carried in presence of H_2O

18 enriched H_2O, the cleavage C-O cleavage path yields glucose containing one atom of ^{18}O. The P-O cleavage is characterized by phosphate containing one atom ^{18}O.

2)Using Isolated Organs/Tissues

The cultivation of isolated organ and tissue of plant which may produce secondary changes in the metabolites

Isolated shoots of plant when placed in asuitable solution or in water

When shoots remain normal for much longer period such shoots often developed roots at the cut end

Isolated leaves can be simillarly maintained rooted leaves have been used in study on nicotina and datura

By these method a large quantity of roots is obtained with relatively small amount of aerial parts

This technique can be extended to cultivation of isolated tissue and cells the use of which afford considerable potentail in the investigation of biogenetic pathway

3)Grafting Methods

The technique of grafting involves the use of grafted plants to study the biosynthesis of alkaloids. This method is also preferred for determining the sites of primary and secondary metabolites of some secondary plant products.

4)Use of Mutant Strains

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A number of mutant strains of microbes that have been developed , lack a n enzyme that blocks their metabolism at a specific stage.

Such microorganisms
may accumulate the intermediate compound just before the blockage, and require an artificial supply of another intermediate (which occurs after the blockage) for their survival

these microorganisms are useful for studying the
biosynthetic pathways of various primary and secondary metabolites.

Utilisation of Radioactive Isotopes

- These are organic compounds whose one or more atom replaced wit
- Radioactive tracers are radioactive isotopes used in biogenetic studie
- Examples of radioisotope tracers used frequently to trace the path of l reactions are hydrogen, carbon, phosphorus, sulphur, and iodine.

Radioactive Isotope	Applications in Medicine
Cobalt-60	Radiation therapy to prevent cancer
Iodine-131	Locate brain tumors, monitor cardiac, liver and thyroid activity
Carbon-14	Study metabolism changes for patients with diabetes, gout and anemia
Carbon-11	Tagged onto glucose to monitor organs during a PET scan
Sodium-24	Study blood circulation
Thallium-201	Determine damage in heart tissue, detection of tumors
Technetium-99m	Locate brain tumors and damaged heart cells, radiotracer in medical diagnostics (imaging of organs and blood flow studies)

Detection and assay of radioactively- labelled compound

When radioactive tracers are used in biogenetic studies adequate methods for the detection and estimation of the label are essential

Rutherford successfully use this method in his early studies on radioactivity and he counted the flashes of light produced by bombardment

made with Alpha particle on a fluorescent screen prepared from zinc sulphide

The traditional unit of radioactivity has been the Curie

The usefulness of the detector was tremendously heightened by the development of the photomultiplier tube which replaced the human eye in recording the scintillation modern instrument are fully automatic and will also measures mixed radistions

For information on the theoretical basis of radioactive isotope utilisation and the regulation governing the use of radioactive substances in Universities and Research establishment The Reader is referred to the standard work and official Publication on these subjects

The SI unit now used for radioactive disintegration rate is becquerel (Bq)

Autoradiography

A technique used for the location of radioactive isotopes in biological and other material is autoradiography in this the specimen is placed in contact with the suitable emulsion and after explosure the latter is developed in the usual manner the resulting radiograph gives the distribution pattern of the radioactive substances in the specimen

Autoradiography is the bio-analytical techniques used to visualise the distribution of radioactive labelling of substances with radioisotopes in a biological sample

It is the method by which radioactive material can be localised within a particular tissue cell, cell organelles or even biomolecules

It is a very sensitive technique and is being used in a wide variety of biological experiments

Autoradiography although used to locate the radioactive substances it can also be used for quantitative estimation by using Densitometer

Reference

1. Trease & Evance 16 th Edition (Page no 157-167)
2. Trease and Evance Pharmacognosy W.C .Evance 15th Edition (Page no 147- 148)
3. Dr.K.Prabhu Pharmacognosy &Phytochemistry -II Thakur Publication Edition 2019 (Page no.33-38)

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