

# **TECHNIQUES FOR ELUCIDATION OF BIOSYNTHETIC PATHWAYS (OR) BIOGENETIC STUDIES**

- The important technique used as the tools for the elucidation of biogenetic pathways (or) biogenetic studies of primary & secondary metabolites are:
  - 1.Tracer techniques.
  - 2.Use of isolated organs & tissues.
  - 3. Grafting methods.
  - 4. Use of mutant strains.

- **1. Tracer technique:** various labeled compounds are used to trace out & study the various precursors & intermediates involved at different stages of biosynthetic path ways.
- **2. Use of isolated organs & tissues:** cultures of the organs tissues & cells growing under controlled aseptic conditions can be used for feeding experiments.
- **3. Grafting:** technique in which 2 cut surfaces of different but closely related plants were grown together.
- **4. Use of mutant strains:** Fungi & micro organisms were used in the studies of biosynthetic studies of the natural products.

# Using isolated organs / Tissues

- In this method, isolated parts of plant (eg., stem, roots, etc ) are used. this technique is used to investigate the synthesis site of a particular compound.
- From the sample plant, shoots or any desired part are isolated and placed in water or in a suitable solution.
- After few days, the plant part undergoes normal metabolism, soon a pathological metabolism starts.

- This technique can be modified by connecting the cut end of the plant part (shoot, root, petal discs etc) to a container of suitable sterile nutrient so that the shoot remains normal for a longer period.
- This entire process is carried out under aseptic conditions.
- Similar method is used for maintaining the isolated leaves.
- A rapid growth occurs under optimum conditions and sub cultures may be produced.

- The biogenetic and growth studies can be performed by adding the selected compounds to the culture as per the need.
- By this method tropane alkaloids are synthesized in the roots of most of the Solanaceae plants.
- This method is useful for investigating various biogenetic pathways, as isolated tissues and cells can be cultivated in this technique.

# **STUDY OF UTILISATION OF RADIOACTIVE ISOTOPES IN THE INVESTIGATION OF BIOGENETIC STUDIES**

Biogenetic study is useful for the isolation of the enzymes involved in the pathways. Under consideration & the in vitro demonstration of their properties & to locate & clone the gene responsible for the synthesis of a particular enzyme.

- Radio active tracers are radio active isotopes used in biogenetic studies.
- These are organic compounds and one or more atoms have been replaced with a radionuclide.
- The radio active tracers are used to investigate the mechanism of chemical reactions by tracing the path followed by the radio isotope from reactants to products.
- Radioisotope tracers used frequently to trace the path of biochemical reactions are hydrogen, carbon, phosphorus, sulphur and iodine.

# TRACER TECHNIQUES

- These radio active isotopes or tracers are used in tracer techniques for investigating the biosynthetic pathways.
- In this technique, a labelled compound is used to investigate the different intermediates and various steps in bio synthetic pathways in plants by tracing them at a given rate and time.
- Useful for the identification of elements existed with identical chemical properties but with different atomic weights which can be used as precursor of plant constituents & used as a markers in biogenetic experiments.



# RADIO ACTIVE TRACERS

- In biological investigations the use of radioactive carbon & hydrogen, sulphur, phosphorous, alkali & alkaline earth metals enables the metabolism of compounds.
- For studies on proteins, alkaloids & amino acids a labeled nitrogen atom may give more specific information than a labeled carbon atom.

- Many compounds which are most conveniently prepared from natural sources are produced by growing chlorella in an atmosphere containing carbon dioxide.
- All the carbon compounds of the organism thus become labeled, each compound possessing a uniform labeling of its carbon atom

# DETECTION AND ASSAY OF RADIO ACTIVELY LABELLED COMPOUNDS

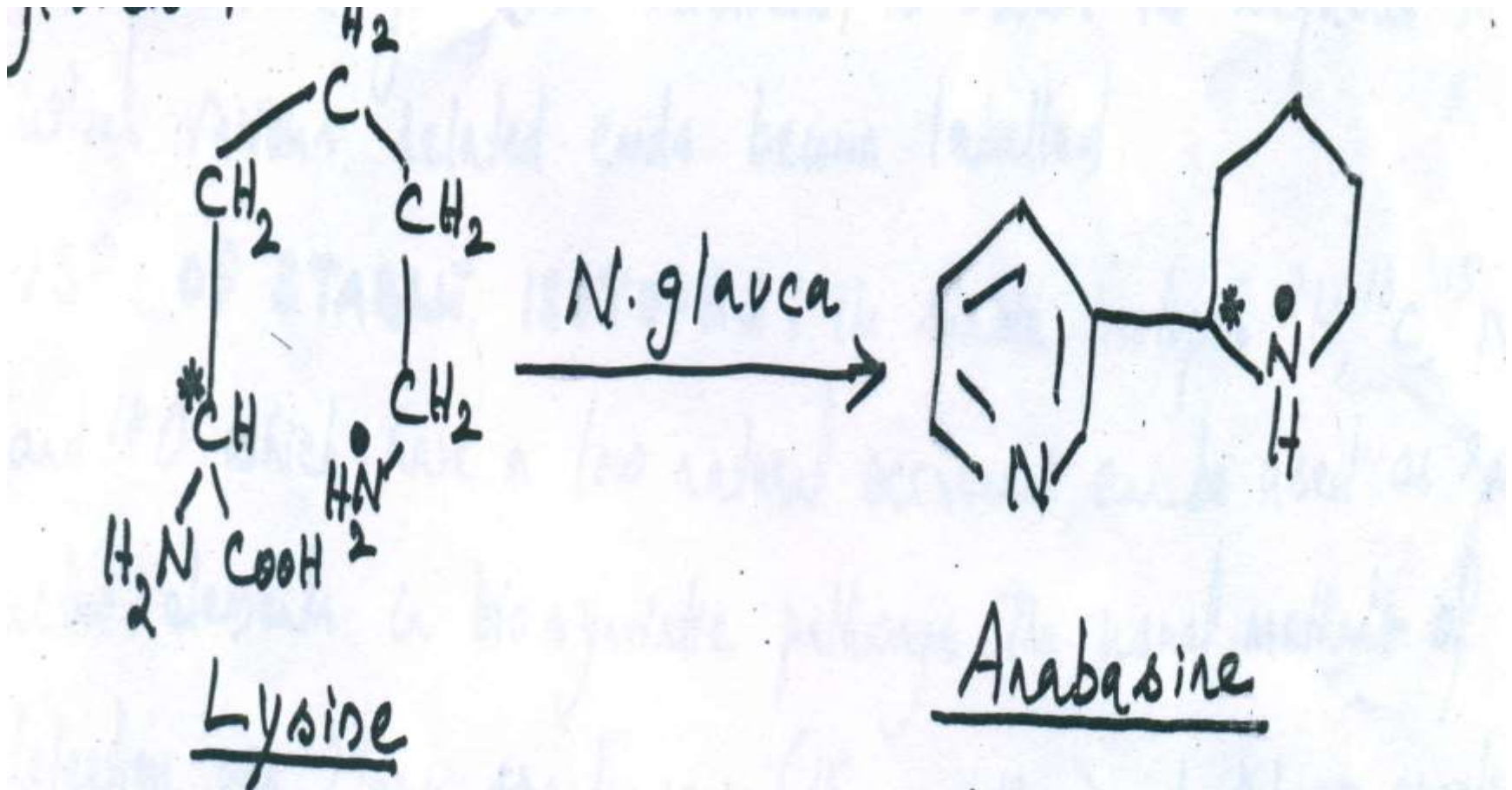
- **LIQUID SCINTILLATION COUNTERS:** is used for the soft and easily absorbed radiation from  $^3\text{H}$  and  $^{14}\text{C}$  labeled compounds (it depends on the conversion of the kinetic energy of a particle into a light by penetrating a suitable luminescent substance).
- Rutherford – studied radio activity, by counting the flashes of light produced by the bombardment with  $\alpha$ -particles on a fluorescent screen prepared from zinc sulphide.

# AUTO RADIOGRAPHY

- Technique used for the identification of the location of radioactive isotopes in biological & other materials.
- The specimen is placed in contact with a suitable emulsion (e.g.: x-ray sensitive film) & the resulting auto radiograph gives the distribution pattern of the radioactive substance in the specimen.
- Eg: T.S of leaf under microscope- similar way radioactive compounds on paper & TLC can be detected.

# PRECURSOR PRODUCT SEQUENCE

- Used for the elucidation of biosynthetic pathways in plants by means of labeled compounds.
- Presumed precursors of the constituent in a labeled form is fed to the plant & after a suitable time the constituent is isolated & purified & its radioactivity is determined.
- Determination of the activity, shows that the labeling was randomly distributed throughout the molecule. For eg : LEETE, used 2 doubly labeled lysines to determine which hydrogen of the lysine molecule was involved in the formation of the piperidine ring of Anabasine in *Nicotiana glauca*.



Incorporation of doubly labeled lysine in to anabasine

# COMPETITIVE FEEDING

Useful in the determination of 2 possible intermediates normally used by the plant.

- **ADMINISTRATION OF PRECURSORS:** To introduce labeled substances in to plants.
- **ROOT FEEDING:** which synthesis the compounds under investigation in the roots.
- **DIRECT INJECTION:** plants with hollow stems (umbelliferae) & to capsules (opium poppy)
- For e.g. –introduction of glucose solution in to chamomile hollow receptacle-micro syringe.

- **SEQUENTIAL ANALYSIS:** used for the investigation of  $^{14}\text{C}$  by growing plants in an atmosphere of  $^{14}\text{C}$ -carbon dioxide & by analyzing the plants at given time intervals, to obtain the sequence in which various related compounds become labeled.
- **USE OF STABLE ISOTOPES:** The stable isotopes which have a low natural occurrence can be used as radioactive element in the biosynthetic pathways. The usual methods of detection are Mass spectroscopy ( ) & NMR spectroscopy ( )



# ISOLATED ORGANS, TISSUES & CELLS

- Used for carrying out experiments with labeled compounds.
- For the determination of the site of synthesis of particular compounds.
- Absolutely uniform plant material can be obtained at all times under regulated & reproducible conditions.
- Potential precursors of the metabolite under investigation can easily be added to the system & samples can be taken repeatedly for analysis.

- Cultures of the organs ,tissues & cells growing under controlled aseptic conditions can be used for feeding experiments.
- The radioactive tracers can be introduced by this process to the parenchymatous tissues of leaves, shoots, roots or other plant structures, on further analysis it provides information's about the incorporation of labeled compounds.

# USE OF MUTANT STRAINS

- Mutant strains like fungi & micro organisms are produced in nature, which lacks one or other enzyme because of which the normal metabolic path ways are greatly affected.
- In such mutant strains metabolites are found at the intermediate stage and needs artificial supply of another intermediate.
- Such mutant strains can be used in the biosynthetic studies of the natural products.
- For eg *Claviceps purpurea*-ultra violet induced strains of produce number of aminoacids of diverse nature.

# GRAFTING METHODS

- Technique in which 2 cut surfaces of different closely related plants placed together & grown.
- Major plant used for grafting is called “STOCK” cut portion of the another plant is called “SCIONS” or “GRAFT”
- Grafting helps for the production of better quality drug. Eg.cinchona, citrus, myristica – by grafting.
- Used for the biosynthetic elucidation of the path ways & used for the biogenesis of secondary metabolites.

- Eg. – the scions of tomato grafted on the stock of datura, shows the accumulation of tropane alkaloid.

-Datura scions grafted with tomato stock – does not show the production of tropane alkaloids, showed only traces of alkaloids.

-Biogenesis of tropane alkaloid is roots & no other organ of datura.