Utilization of radioactive isotopes in the investigations of Biogenetic studies (Tracer Techniques)

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Radioactive tracers

- **Radioactive tracers are fundamental tools in the investigation of biological processes:**
  - Radioactive tracers have been used for the investigation of biological processes since 1930.
  - The tracer isotope may occur as the free element, or incorporated into chemical compounds.
- **The basic principles in biological tracer techniques may be formulated in this way:**
  - The biological system under investigation is sensitive to the chemical nature of the elements, but it cannot distinguish between different isotopes of the same element.
  - The individual isotopes differ by their atomic mass, and by some of them being radioactive

- This physical difference makes it possible to detect the individual isotopes of an element by physical detection methods.
- In order to be useful as a tracer, it must be possible to identify and determine the amount of a specific isotope independently of the presence of other isotopes of the same element.
- When working with radioactive isotopes as tracers, it is the ionizing radiation emitted during radioactive decay, which forms the basis for identification and quantification.
- Methods for the detection of ionizing radiation are extremely sensitive, and radiotracer work therefore requires only very small amounts of radioactively labelled compounds.

Tracer techniques

- A technique which utilizes a labeled compound to trace or find out the different intermediates and various steps involved in biosynthetic process in plant at given rate and given time.
- When these labeled compounds are administered into the plants, they become a part of general metabolic pool and undergo reactions characteristics to the metabolism of that particular plant.
- Tracer techniques in the classical sense comprise a set of methods based on the application of stable or unstable (radioactive) isotopes of an element as markers for the naturally occurring form of the element.

Significance of tracer techniques

- Tracing of biosynthetic pathway by incorporating radioactive isotopes into the precursor or starting material.
  - e.g. By in corporation of C^{14} to phenylalanine, the biosynthesis of cynogenetic glycoside, prunacin can be traced.
- Location and quantity of the compound can be determined in biological system.
- Radioactive tracer methods are used both in research and routine analytical methods.

Different trace element for different studies

- For studies on Proteins, Alkaloid and Amino acid: Nitrogen atom gives more specific information than carbon atom.
- For studies on glycosidic linkage: O, N, S and C atoms.
- For studies on Terpenoids: O atom.

Basic steps involved in tracer techniques

- Preparation of labeled compound.
- Incorporation of labeled compound to tissue system.
- Separation or isolation of labeled from tissue system.
- Determination of nature of metabolites in various biochemical fraction

Preparation of labeled compound

- The labeled compounds may be prepared by use of radioactive isotopes and stable isotopes.
- Radioactive isotopes: C^{14}, H^{3},S^{35},P^{32},I^{131},Co^{60}.
- Stable isotopes: H^{2}, C^{13}, N^{15}, O^{18}.

Properties of some radioactive isotopes

Natural	Radioactive	Radiation	Half life
C^{12}	C^{14}	β	5760 yrs
H^{1}	H^{3}	β	12.5 yrs
S^{32}	S^{35}	β	871 days
P^{31}	P^{32}	β	14.3 days
Cl^{35}	Cl^{36}	β	4.4×10^{5} yrs
I^{127}	I^{131}	β, γ	8 days
Co^{59}	Co^{60}	β, γ	5.3 days

Criteria for selection of trace elements

- The starting concentration of trace element must be sufficient enough to withstand dilution in the course of metabolism.
- For proper labeling the physical and chemical nature of the compound must be known.
- Half life of the tracer isotope should be sufficiently long.
- The labeled compound should not be damage the tissue system

- In biological investigations the use of radioactive isotopes enables the metabolism of compounds to be followed in living organisms for detection and estimation of soft and easily absorbed radiation from labeled compounds.

- The instrument of choice to detect the properties of metabolites is scintillation Counter, GM-Counter, Ionization chamber, Mass Spectroscopy, NMR etc.

- e.g. Growing chlorella in an atmoshphere containing $^{14}CO_2$.

- Tritium labeling is effected by catalytic exchange (Pt catalyst) in aqueous media by hydrogenation of unsaturated compounds with tritium gas.

Introduction of labeled compound to tissue system

- There are six methods used to incorporate labeled compounds to tissue system:
  - Root feeding
  - Stem feeding
  - Direct injection
  - Infiltration
  - Floating method
  - Spraying technique

Root feeding

- The plant in which roots are biosynthetic sites; this method is preferred e.g. Tobacco.

- In this type of experiment the plant are cultivated hydroponically to avoid microbial contamination.

Stem feeding

- The presence of root don’t require for biosynthesis.

- In this method substrate can be administered through the cut ends of stem immersed in a solution. For latex containing plant; this method is not suitable

Direct injection

- This method is applicable to the plant with hollow stem. e.g. Umbelliferae and capsules bearing plant (Opium poppy).

Infiltration : Wick feeding

- When it is desired to carryout feeding on plant rooted in soil or other support without disturbing the root , wick feeding is applicable.

Floating method:

- When small amount of material is available, floating method is used. In this method leaf disc or chopped leaves are floating on the substrate solution.
- This technique is also used in conjugation with vacuum infiltration to remove gases.

Spray technique

- In this method compound have been absorbed after being sprayed on leaves in aqueous solution. e.g. Steroides

Separation or isolation of labeled compound or metabolite

- Depending on the nature of drug and its source different method of extraction is employed:
  - Soft and fresh tissue: Infusion, Maceration.
  - Hard tissue: Decoction, Hot percolation.
  - Unorganized drug: Maceration with adjustment choice of solvent for extraction.
  - Fat and Oil: Non-polar solvent
  - Alkaloid, Glycoside, Flavanoides: Slightly polar solvent
  - Plant phenol: Polar solvent

- Fractional crystallization, partition, column chromatography also used as separation technique.

Determination of nature of metabolites

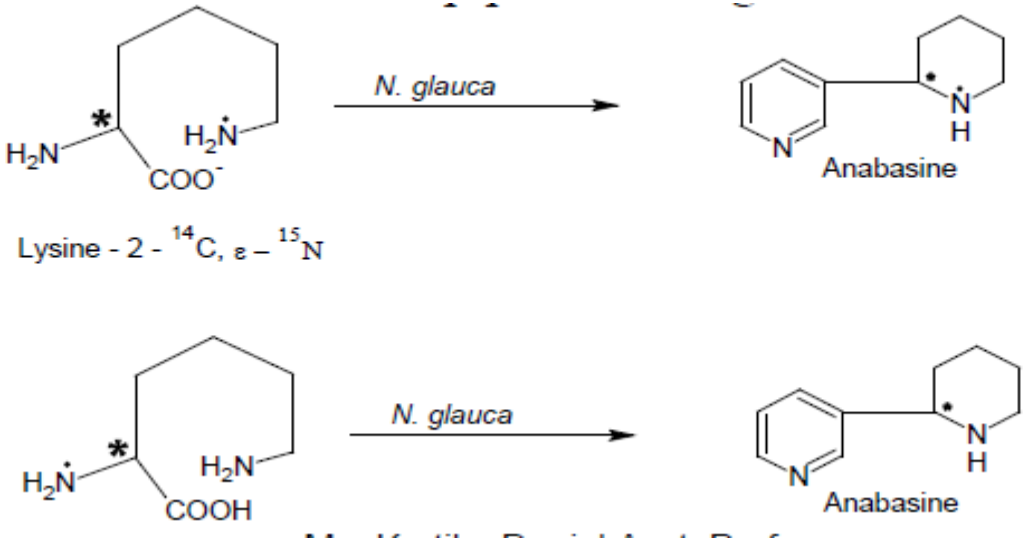
- Depending on nature of isotopes various instrumentation techniques are used for determination of chemical nature of intermediate and final product.
- For radioactive isotopes:
  - GM-Counter
  - Scintillation or liquid scintillation counter
  - Ionization chamber

These entire instruments characterized the nature of radiation. Basically it depends upon the conversion of kinetic energy of particle into fleeting pulse of light as a result of its penetration into a suitable luminescent medium.

- For stable isotopes:
  - Mass spectroscopy: gives molecular peaks depending on mass/charge ratio.
  - NMR: gives nature of carbon and proton.

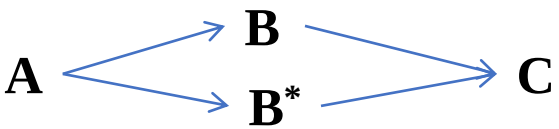
- Precursor-product sequence

- For elucidation of biosynthetic pathways in plants by means of labeled compounds, the precursor product sequence method is used.
- In this method presumed precursor of the constituent is under investigation in a labeled form is fed to plant and after a particular time the constituent is isolated, purified and its radioactivity is determined.
- This method is extensively applied to the biogenesis of morphine and ergot alkaloid.

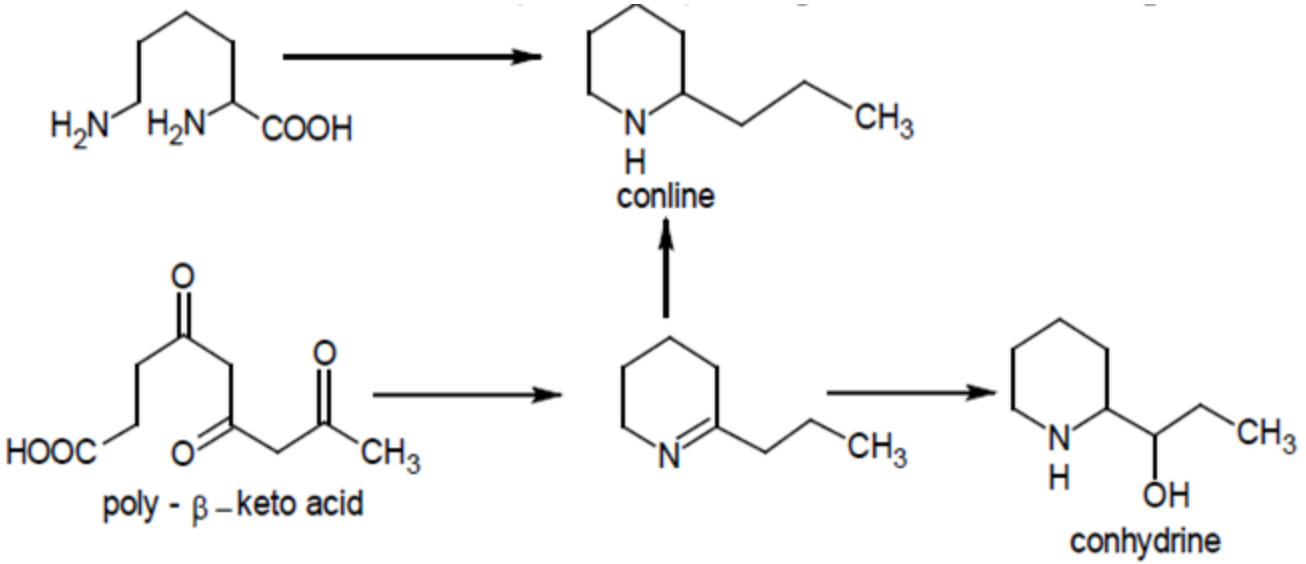


• Competitive feeding

- This method is normally used to determine two possible intermediates in the plants.



- Competitive feeding can distinguish whether B or B^* is the normal intermediate in the formation of C from A.
  - Biosynthesis of Hemlock alkaloids (Coniine and Conhydrine)



- **Sequential Analysis**
  - The principle of this method of investigation is to grow plants in the atmosphere of $^{14}CO_2$ and by analysis of plants which various related compounds becomes labeled from the others rejected.
    - $^{14}CO_2$ and sequential analysis has been very successfully used in the elucidation of the path of carbon in photosynthesis.
    - Determination of sequential formation of menthol in *Mentha piperita*

APPLICATION OF TRACER TECHNIQUE

1. Study of squalene cyclization by use of ^{14}C, ^{3}H labelled mevalonic acid.
2. Interrelationship among 4 – methyl sterols & 4, 4 dimethyl sterols, by use of ^{14}C acetate.
3. Terpenoid biosynthesis by chloroplast isolated in organic solvent, by use of 2- ^{14}C mevalonate.
4. Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin.
5. Origin of carbon & nitrogen atoms of purine ring system by use of ^{14}C or ^{15}N labelled precursor.
6. Study of formation of scopoletin by use of labelled phenylalanine.
7. By use of ^{45}Ca as tracer, - found that the uptake of calcium by plants from the soil. (CaO & $CaCO_2$).
8. 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.