BIOANALYTICAL TECHNIQUES; COURSE CODE- BTA050

Unit 4: Radioactivity

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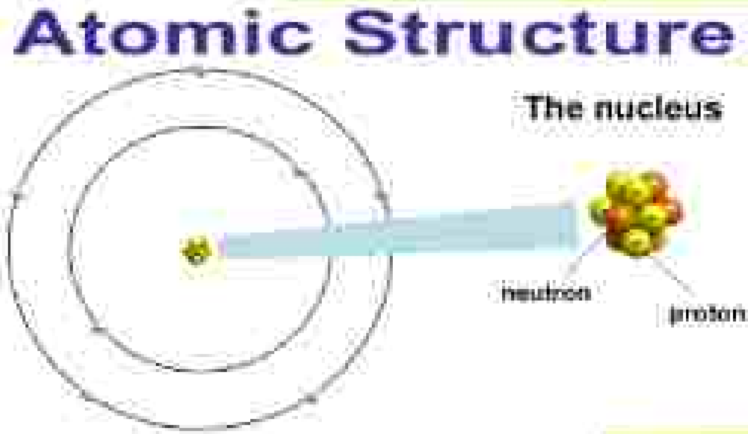
- Isotopes, stable Isotopes
- Radioactive decay, Rate of radioactive decay.
- Measurement of Radioactive decay: Geiger-Muller counter, Solid & Liquid scintillation counters. Radiation dosimetry, Cerenkov radiation, Autoradiography
- Measurement of stable isotopes- Falling drop method.
- Applications of isotopes in biochemistry
- Radiotracer techniques
- Distribution studies
- Isotope dilution technique
- Metabolic studies
- Clinical application
- Radioimmunoassay

Structure and Properties of the Nucleus, Radioactivity

- Its an emission of particles or electromagnetic radiation from the nucleus.
- A nucleus is made of **protons and neutrons**
- **Neutron + proton : Nucleons**
- **Different nuclei are referred as Nuclides.**
- **A proton is positively charged; denoted by Z; it represented atomic number. Its mass is: $m_p = 1.67262 * 10^{-27}$ kg**
- **A neutron is electrically neutral. denoted by N; it $m_n = 1.67493 * 10^{-27}$ kg**
- The sum of protons and neutrons in a given nucleus is the **mass number** (*A* ; **Atomic mass number**).
  - **A = Z+N**

X is the chemical symbol for the element; it contains the same information as Z but in a more easily recognizable form.

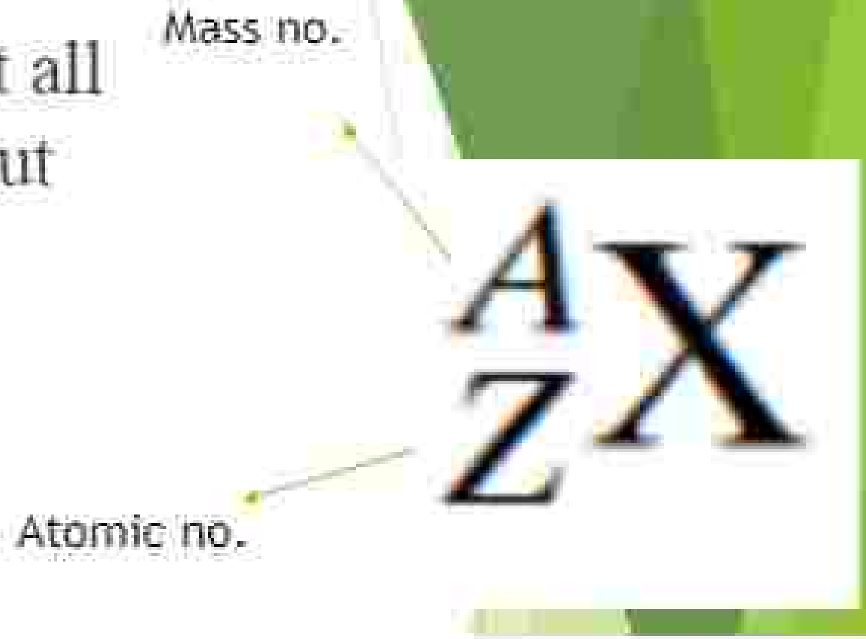


$${}^{A}_{Z}X$$

Isotopes

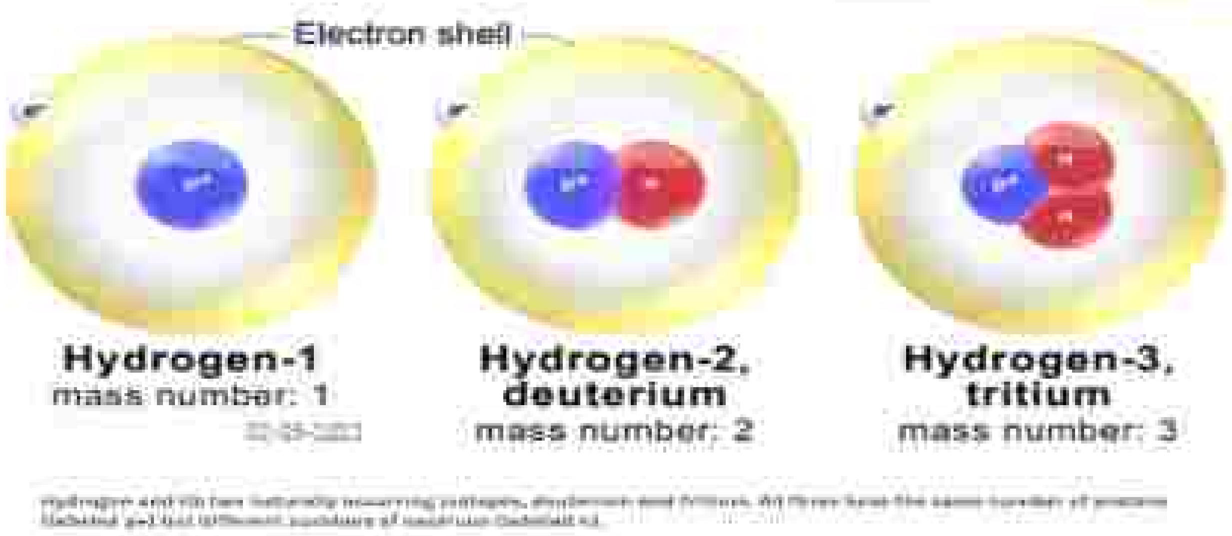
- Isotopes are members of a family of an element that all have the same number of protons (Z; atomic no.) but different numbers of neutrons (N; mass no.).

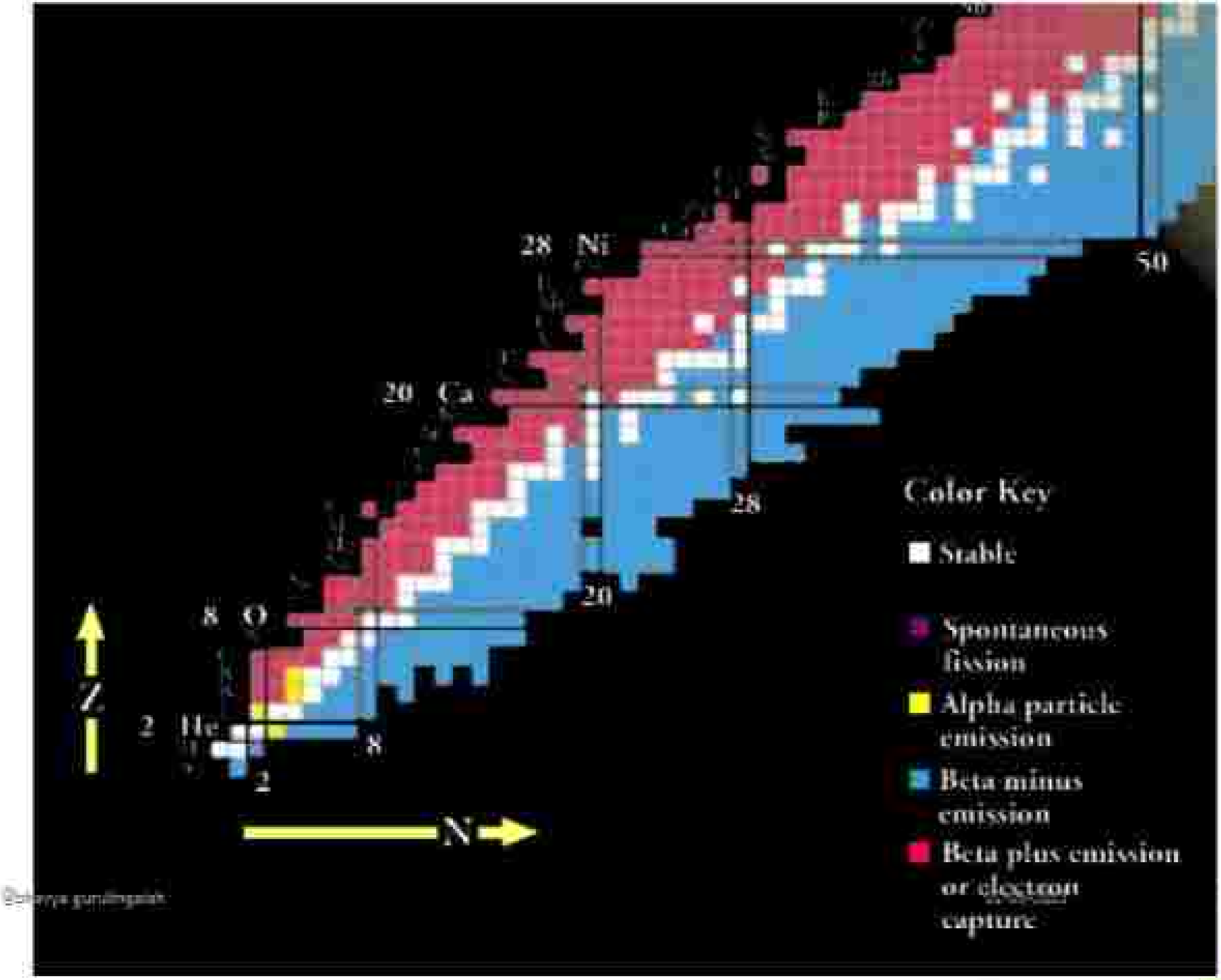
$^{A}_{Z}\text{Element} \quad ^{12}_{6}\text{C} \quad ^{14}_{6}\text{C} \quad ^{16}_{8}\text{O} \quad ^{18}_{8}\text{O}$



Isotopes of Hydrogen are:

^{1}H: stable, ^{2}H: Deuterium and ^{3}H: Tritium




28 Ni
20 Ca
8 O
2 He
Z
N
2
8
20
28
50
Color Key
■ Stable
● Spontaneous fission
■ Alpha particle emission
■ Beta minus emission
■ Beta plus emission or electron capture

Radioactivity

Towards the end of the 19th century, minerals were found that would darken a photographic plate even in the absence of light.

This phenomenon is now called radioactivity.

Marie and Pierre Curie isolated two new elements that were highly radioactive; they are now called polonium and radium.

Atomic Stability and Radiation

- the ratio of neutrons (N) to protons (P) : determines the stability of an isotope.
- Stable isotopes for elements with low atomic numbers tend to have an equal number of neutrons and protons..
- Stability for elements of higher atomic numbers requires more neutrons.
- Unstable isotopes are called **radioisotopes.**
- **Radioisotopes** become stable isotopes by the process of **radioactive decay**
- **In radioactive processes, particles or electromagnetic radiation are emitted from the nucleus. The most common forms of radiation emitted have been traditionally classified as alpha (α), beta (β), and gamma (γ) radiation.**

Radioactivity

Radioactive rays were observed to be of three types:

1. Alpha rays, which could barely penetrate a piece of paper
2. Beta rays, which could penetrate 3 mm of aluminum
3. Gamma rays, which could penetrate several centimeters of lead

We now know that alpha rays are helium nuclei, beta rays are electrons, and gamma rays are electromagnetic radiation.

Properties of different types of radiations

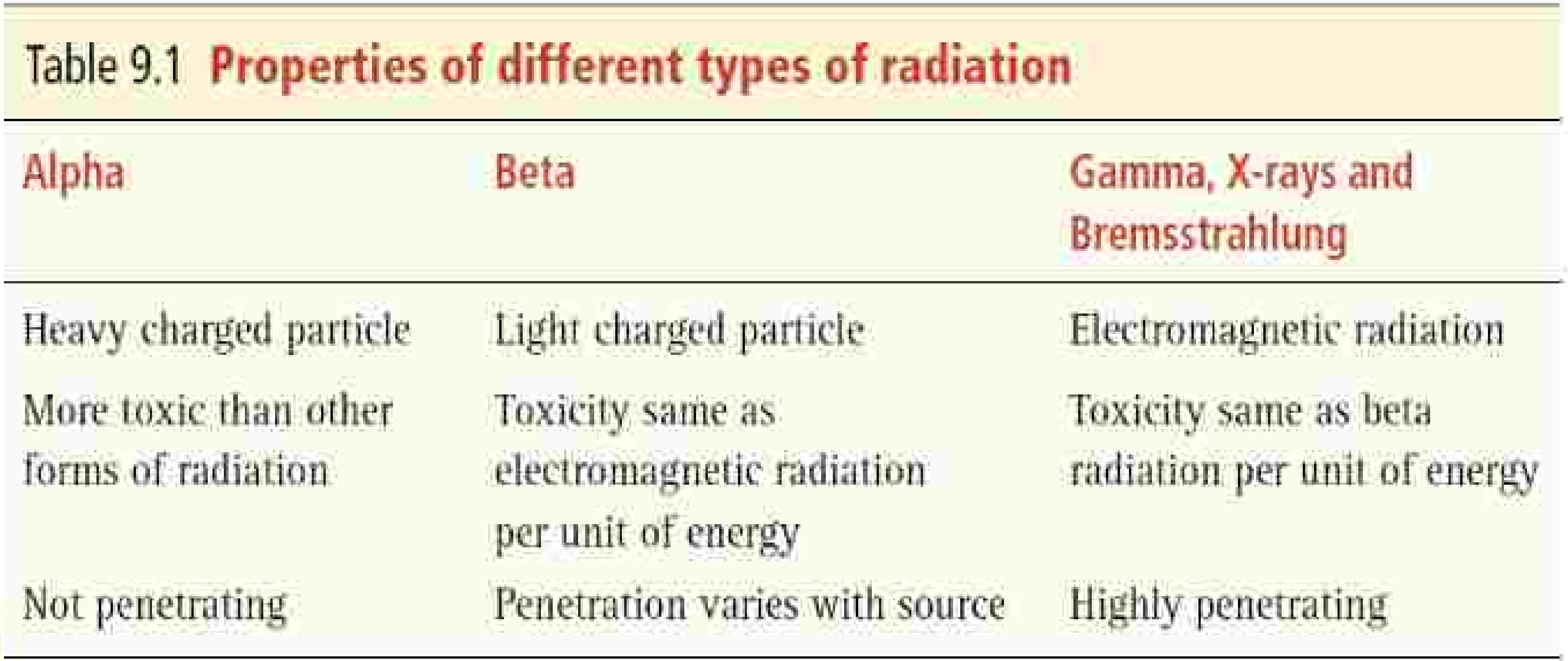


Table 9.1 **Properties of different types of radiation**

Alpha	Beta	Gamma, X-rays and Bremsstrahlung
Heavy charged particle	Light charged particle	Electromagnetic radiation
More toxic than other forms of radiation	Toxicity same as electromagnetic radiation per unit of energy	Toxicity same as beta radiation per unit of energy
Not penetrating	Penetration varies with source	Highly penetrating

Radioactive decay and rate of Radioactive decay

- During the **radioactive decay, it** induces changes to occur in the atomic nucleus, and particles and/or electromagnetic radiation are emitted – to become an stable Isotopes.
- Radioactive decay is a spontaneous process and measured as disintegrations per second (d.p.s.) or per minute (d.p.m.)
- Radioactive decay is a nuclear event
- Radioactive decay is the **electron volt** (eV) or (MeV)
- Majority of isotopes, the term million or mega electron volts (MeV) is more applicable.
- Isotopes emitting *α-particles are normally the most energetic, falling in the range 4.0 to 8.0MeV,*
- The *$\beta-$ and γ -emitters* generally have decay energies of less than 3.0MeV.
- The higher the energy of radiation the more it can penetrate matter and the more hazardous it becomes.
- Rate of radioactive decay: Radioactive decay is a spontaneous process and measured as disintegrations per second (d.p.s.) or per minute (d.p.m.).
- Thus defined by the rate constant λ.

Rate of Radioactive decay

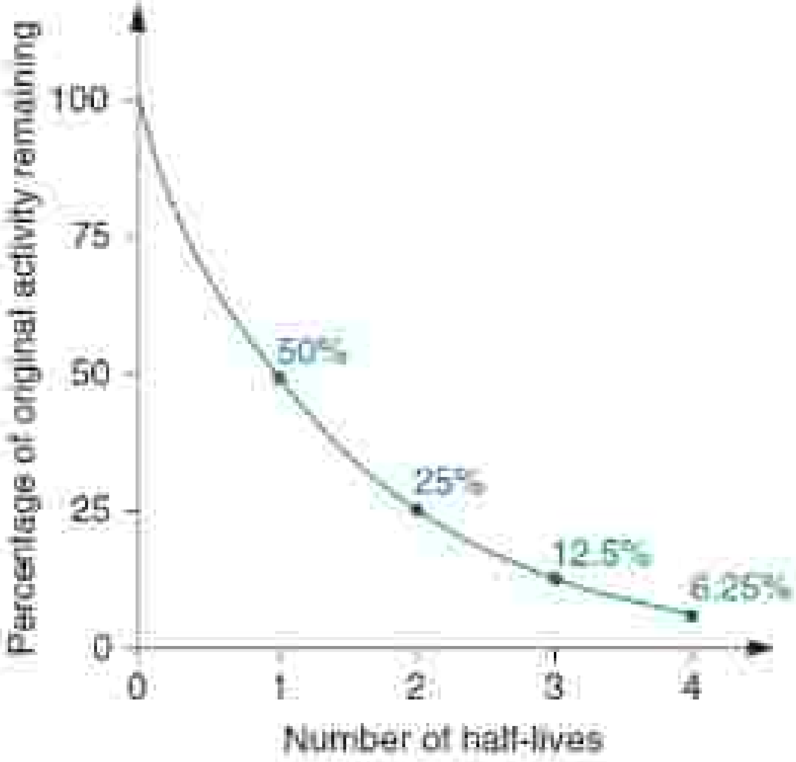


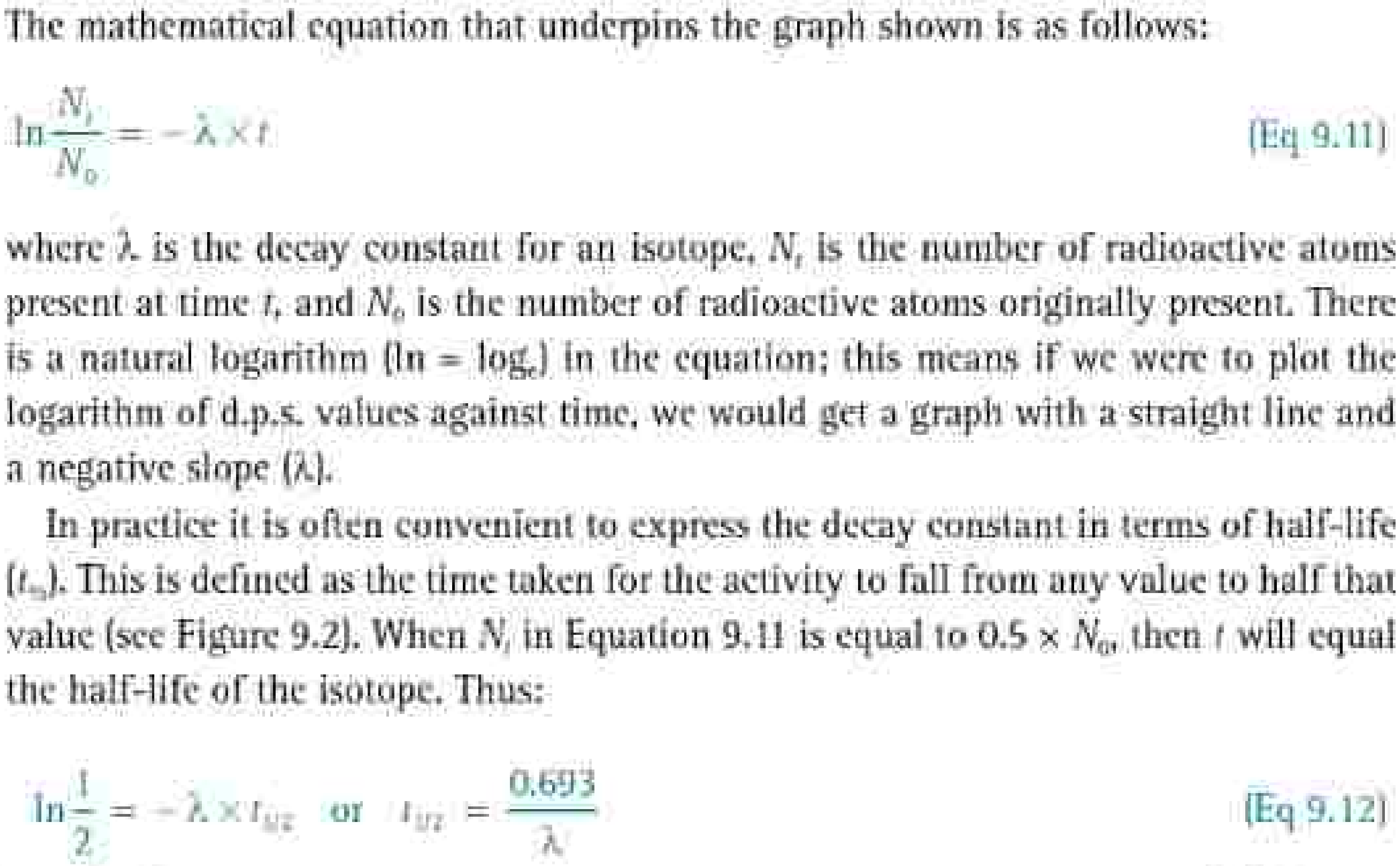
Figure 9.2 Demonstration of the exponential nature of radioactive decay.

- the number of atoms *N* is always decreases.
- This results in the rate of decay decreases with time.
- It follows first-order kinetics and a plot of radioactivity against time thus yields a so-called **exponential decay curve.**
- Half-life is the length of time it takes for half of the radioactive atoms of a specific radionuclide to decay. Denoted by $t_{1/2}$
- **λ denotes rate constant.**

$$t_{1/2} = \frac{0.693}{\lambda}$$

If T i.e. is the mean lifetime of the parent nuclei is given then $t_{1/2} = 0.693T$.

$T_{1/2}$ derivation using exponential decay curve



The mathematical equation that underpins the graph shown is as follows:

$$\ln\frac{N_t}{N_0} = -\lambda \times t \qquad \text{(Eq 9.11)}$$

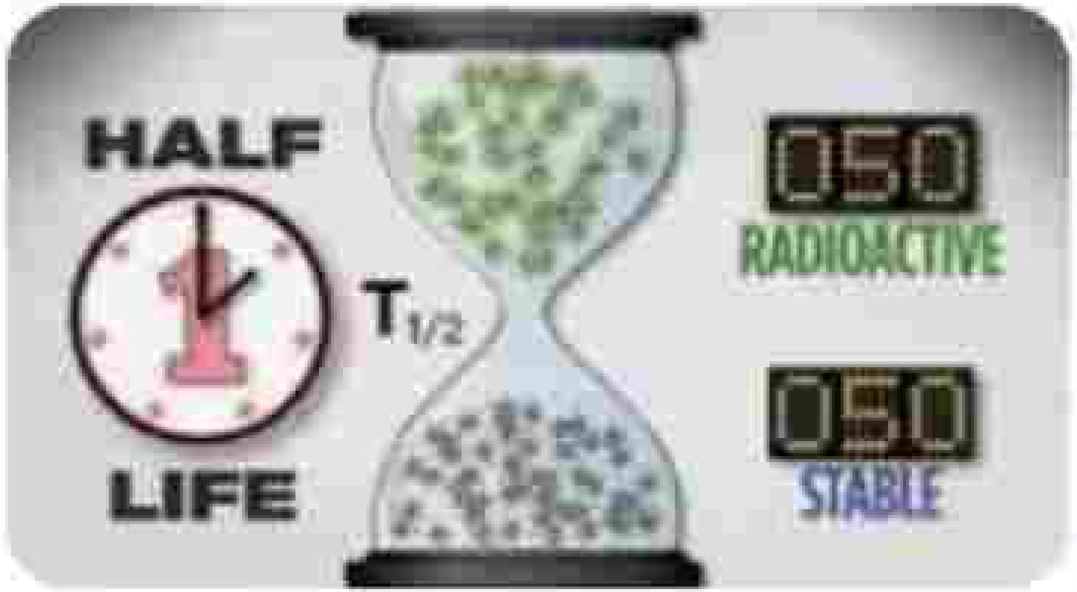
where λ is the decay constant for an isotope, N_t is the number of radioactive atoms present at time t, and N_0 is the number of radioactive atoms originally present. There is a natural logarithm ($\ln = \log_e$) in the equation; this means if we were to plot the logarithm of d.p.s. values against time, we would get a graph with a straight line and a negative slope (λ).

In practice it is often convenient to express the decay constant in terms of half-life ($t_{1/2}$). This is defined as the time taken for the activity to fall from any value to half that value (see Figure 9.2). When N_t in Equation 9.11 is equal to $0.5 \times N_0$, then t will equal the half-life of the isotope. Thus:

$$\ln\frac{1}{2} = -\lambda \times t_{1/2} \quad \text{or} \quad t_{1/2} = \frac{0.693}{\lambda} \qquad \text{(Eq 9.12)}$$

Radioactive decay

a. **Decay by Negatron Emission (β^{-})**
b. **Decay by Positron Emission (β^{+})**
c. **Decay by alpha particle Emission (α^{-})**
d. **Electron Capture and decay by emission of γ rays**



a. Decay by Negatron Emission (β^-)

- Neutron is converted to a proton by the ejection of a negatively charged beta (β)-particle called a **negatron** (β^-).

$$\text{neutron} \longrightarrow \text{proton} + \text{negatron}$$

- Negatron is an electron.
- Result of negatron emission, the nucleus loses a neutron, but gains a proton.
- Mass number *A* remains constant.
- Example carbon-14. An isotope frequently used in biological work that decays by negatron emission is carbon-14, which decays to nitrogen

$$^{14}_{6}C \longrightarrow {}^{14}_{7}N + \beta^-$$

- Plays an important role in biological molecules studies.
- Example use of ^{14}C, ^{3}H, : labelling organic molecule
- ^{32}P or ^{33}P in labelling DNA (nucleic acid molecular biology).

b. Decay by Positron Emission (β^+)

- Some isotopes decay *by emitting positively charged β -particles*, referred to as ***positrons*** (β^+).

$$\text{proton} \longrightarrow \text{neutron} + \text{positron}$$

- Positrons are extremely unstable.
- They interact with electrons and are annihilated.
- The mass and energy of these two particles are converted to two γ-rays emitted at 180° to each other.
- As a result of positron emission, the *nucleus loses a proton and gains a neutron, the mass number A stays the same*.
- example of an isotope decaying by positron emission is sodium-22, which decays to neon.

$$^{22}_{11}\text{Na} \longrightarrow {}^{22}_{10}\text{Ne} + \beta^+$$

- Detection by γ –radiation detector.
- *Importance*

PET scan :

To spectacular effect in brain scanning with the technique **positron emission tomography** (PET scanning) used, for example, to identify active and inactive areas of the brain.

This is also called as Beta decay (which occurs when a nucleus emits an electron).

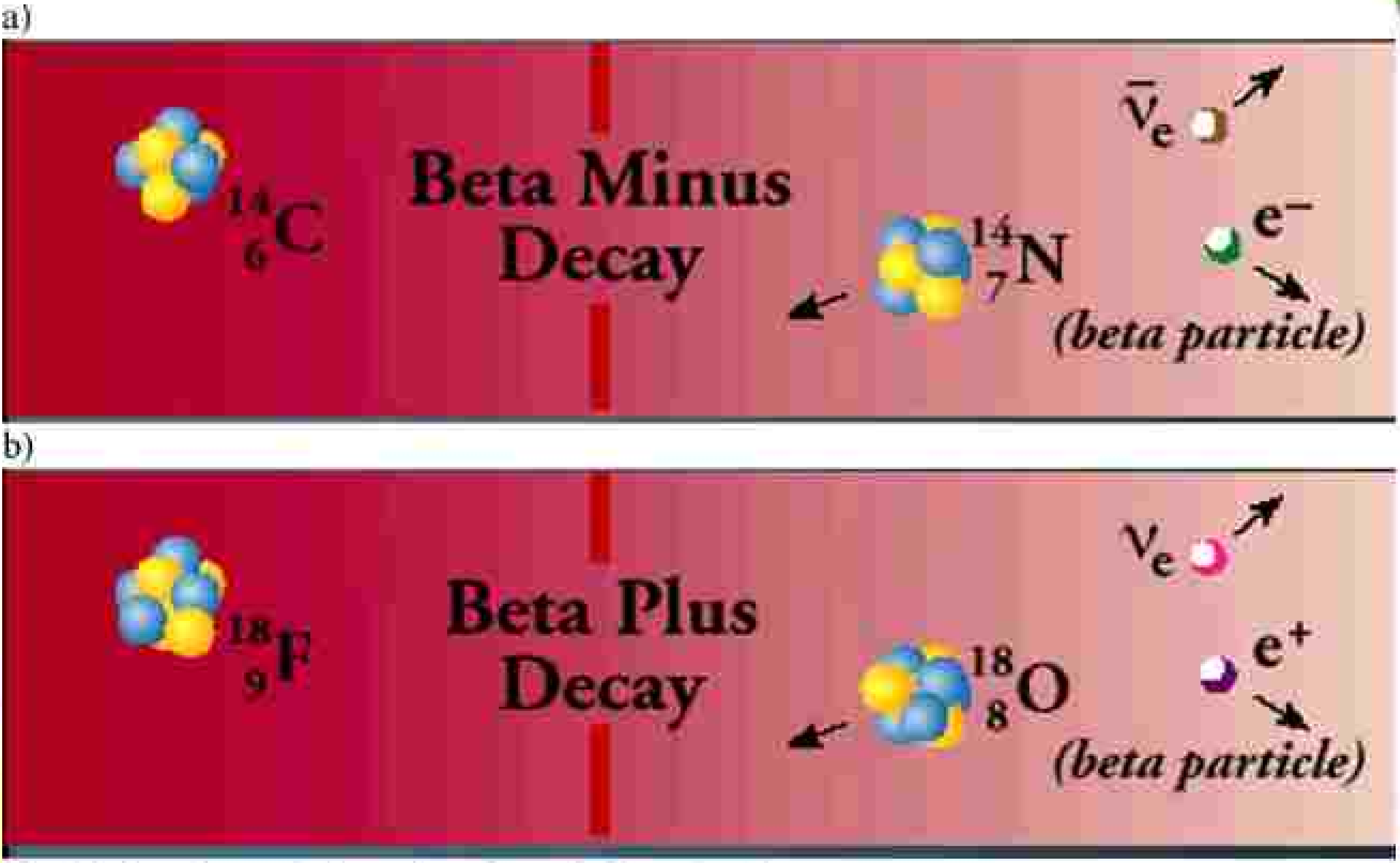


Fig. 3-5. Beta decays. a) Beta-minus decay. b) Beta-plus decay.

c. Decay by alpha particle Emission (α^+)

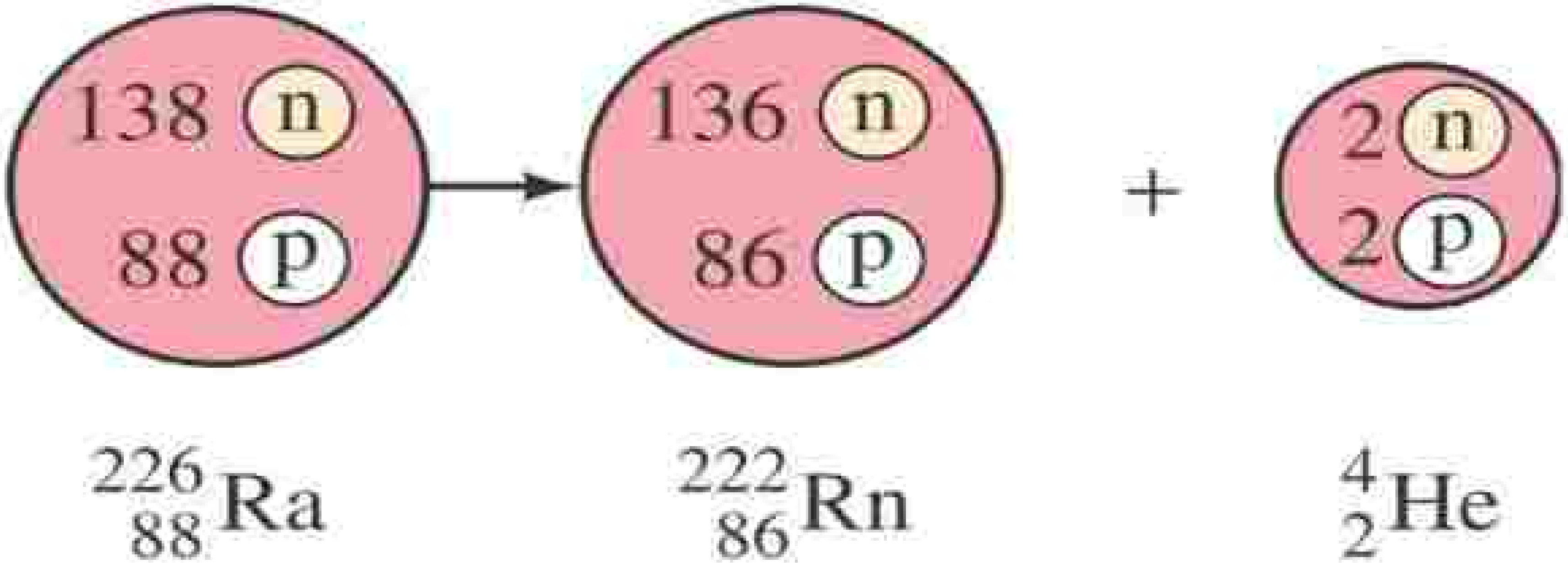
- Isotopes of elements with high atomic numbers (have too large a proton to neutron ratio) frequently decay by emitting **alpha (α)-particles** .
- The nucleus emits a ^{4}He nucleus, an alpha (**α**) particle.
- An alpha particle, with its two protons and two neutrons, is a very stable configuration of particles.
- Alpha radiation reduces the ratio of protons to neutrons in the parent nucleus, bringing it to a more stable configuration.
- i.e. a decrease in atomic number (Z) of 2 and a decrease in the mass number (A) of 4.
- The atomic number changes, so the original (or parent) atoms and the decay-product (or daughter) atoms are different elements and therefore have different chemical properties.
- Nuclei, which are more massive than lead, frequently decay by this method.
- Normally found in instruments such as scintillation counters and smoke alarms.

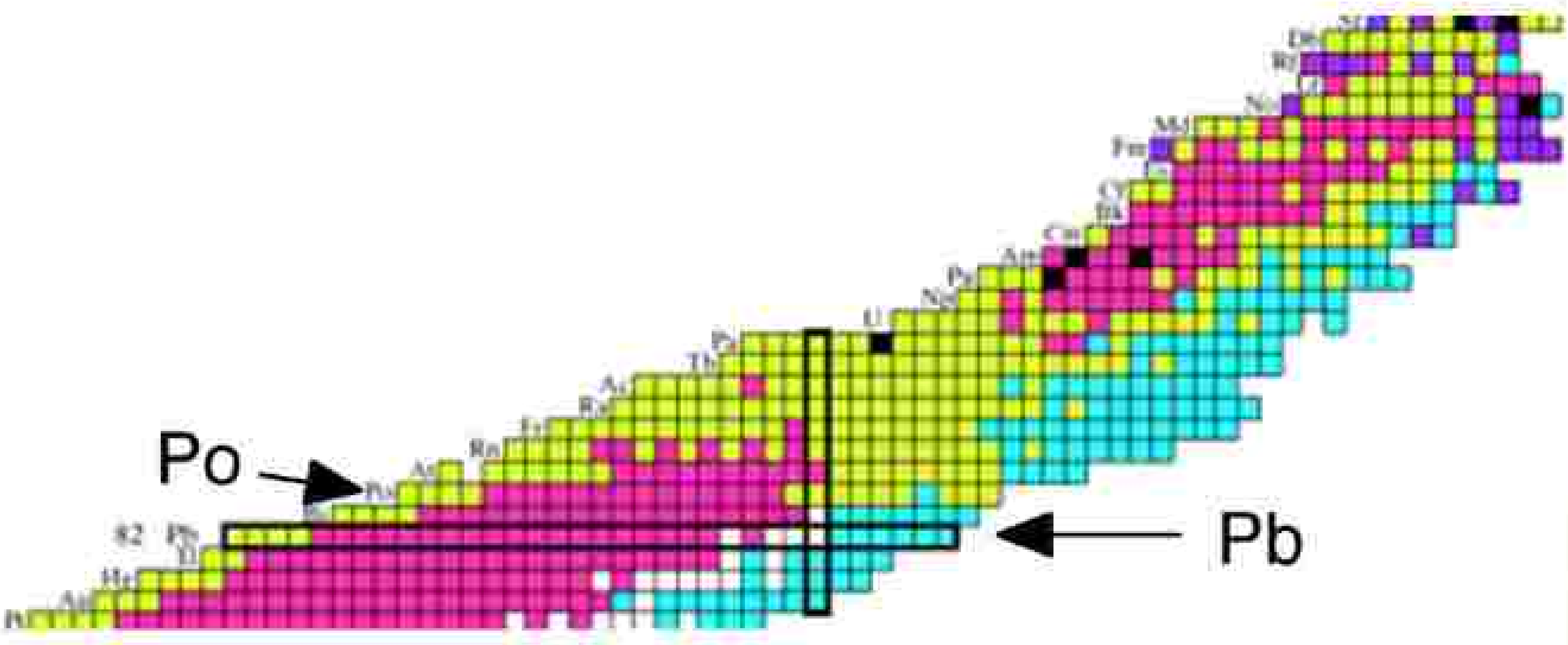
Alpha Decay

Example of alpha decay:

Radium-226 will alpha-decay to radon-22

$$^{226}_{88}\text{Ra} \rightarrow \, ^{222}_{86}\text{Rn} + \, ^{4}_{2}\text{He}$$




Po
Pb
82
Pt
Au
Hg
Tl
Pb
Bi
Po
At
Rn
Fr
Ra
Ac
Th
Pa
U
Np
Pu
Am
Cm
Bk
Cf
Es
Fm
Md
No
Lr
Rf
Db
Sg

d. Electron Capture and decay by emission of γ rays

- A proton captures an electron near the nucleus.
- The proton becomes a neutron and electromagnetic radiation (γ-rays/photons) is released.
- Example: Iodine-125 decays to tellurium-125 by an electron capture mechanism.

$$^{125}_{53}\mathrm{I} \longrightarrow {}^{125}_{52}\mathrm{Te} + \gamma\text{-ray}$$

- γ-**Rays** constitute electromagnetic radiation at a very short wavelength, and therefore possess the same sort of energy as **X-rays**
- In some cases α- and β-particle emission also gives rise to γ –rays.
- γ -radiation has low ionizing power, but high penetration.
- In diagnosis and treatment of thyroid disorders (thyroid hormone contains iodine); it decays to xenon-131.
- The γ emission has a very short **half-life.**

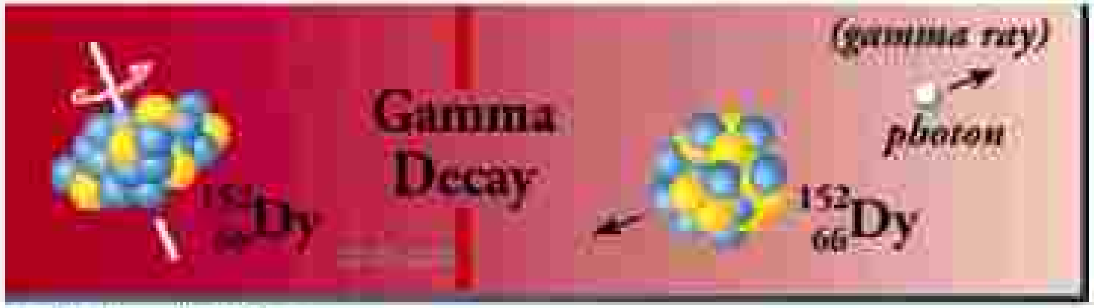
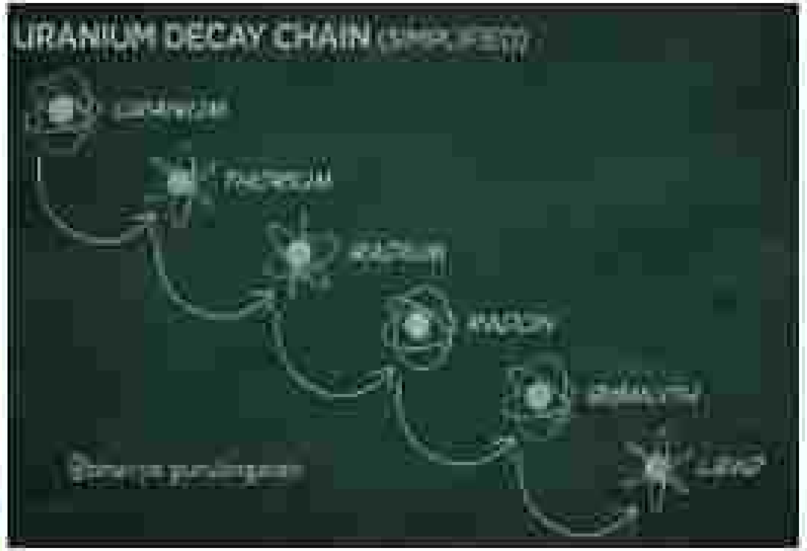


Fig. 3-6. A gamma (γ) decay

Radioactive Decay Chains

- Some radionuclides go through a series of transformations before they reach a stable state.
- several types of radioactive atoms are generated. This is called a decay chain.
- Example:

When uranium-238 decays, it produces several isotopes of: Thorium, Radium Radon, Bismuth and finally stable state – Pb.



Radium-226, for example, decays by -emission to radon-222, which is itself radioactive. This begins a complex decay series, which culminates in the formation of lead-206.

$$^{226}_{88}\mathrm{Ra} \longrightarrow {}^{4}_{2}\mathrm{He}^{2+} + {}^{222}_{86}\mathrm{Rn} \longrightarrow \longrightarrow \longrightarrow {}^{206}_{82}\mathrm{Pb}$$

Units of Radioactivity

- SI system : becquerel (Bq)
- It is one **disintegration per second (d.p.s)**
- No SI system unit still in use is **curie** (Ci).
- One Ci: defined as the quantity of radioactive material in which the number of d.p.s. is the same as that in 1 g of radium, that is 3.7 × 10 10 s −1 (or 37 GBq).
- In biological work its represented as microcurie (Ci) and millicurie (mCi)
- *Detected decays* are referred to as **counts** and the corresponding rate is thus reported as counts per second (***c.p.s.***).

Properties of Radioisotopes commonly used in the biological sciences

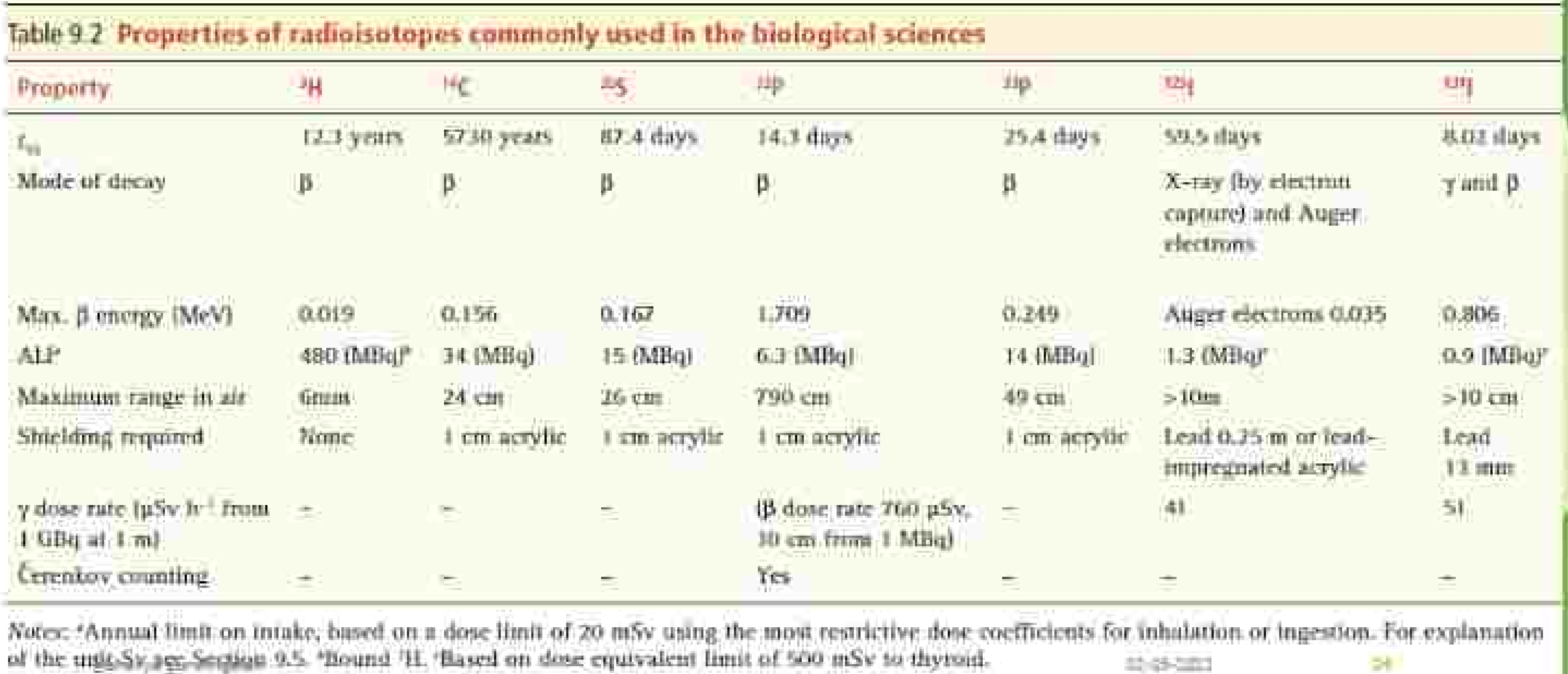


Table 9.2 **Properties of radioisotopes commonly used in the biological sciences**

Property	^{3}H	^{14}C	^{35}S	^{32}P	^{33}P	^{125}I	^{131}I
$t_{1/2}$	12.3 years	5730 years	87.4 days	14.3 days	25.4 days	59.5 days	8.02 days
Mode of decay	β	β	β	β	β	X-ray (by electron capture) and Auger electrons	γ and β
Max. β energy (MeV)	0.019	0.156	0.167	1.709	0.249	Auger electrons 0.035	0.806
ALI[a]	480 (MBq)[b]	34 (MBq)	15 (MBq)	6.3 (MBq)	14 (MBq)	1.3 (MBq)[c]	0.9 (MBq)[c]
Maximum range in air	6mm	24 cm	26 cm	790 cm	49 cm	>10m	>10 cm
Shielding required	None	1 cm acrylic	1 cm acrylic	1 cm acrylic	1 cm acrylic	Lead 0.25 m or lead-impregnated acrylic	Lead 13 mm
γ dose rate ($\mu Sv\ h^{-1}$ from 1 GBq at 1 m)	–	–	–	(β dose rate 760 μSv, 30 cm from 1 MBq)	–	41	51
Čerenkov counting	–	–	–	Yes	–	–	–

Notes: [a] Annual limit on intake, based on a dose limit of 20 mSv using the most restrictive dose coefficients for inhalation or ingestion. For explanation of the unit Sv see Section 9.5. [b] Bound ^{3}H. [c] Based on dose equivalent limit of 500 mSv to thyroid.

MEASUREMENT OF RADIOACTIVITY

- Three commonly used methods of detecting and quantifying radioactivity.
- The ionisation of gases, on the excitation of solids or solutions, and imaging techniques.
- for example autoradiography (the use of photographic emulsions and films) or phosphor imaging.

Detection of Radiation

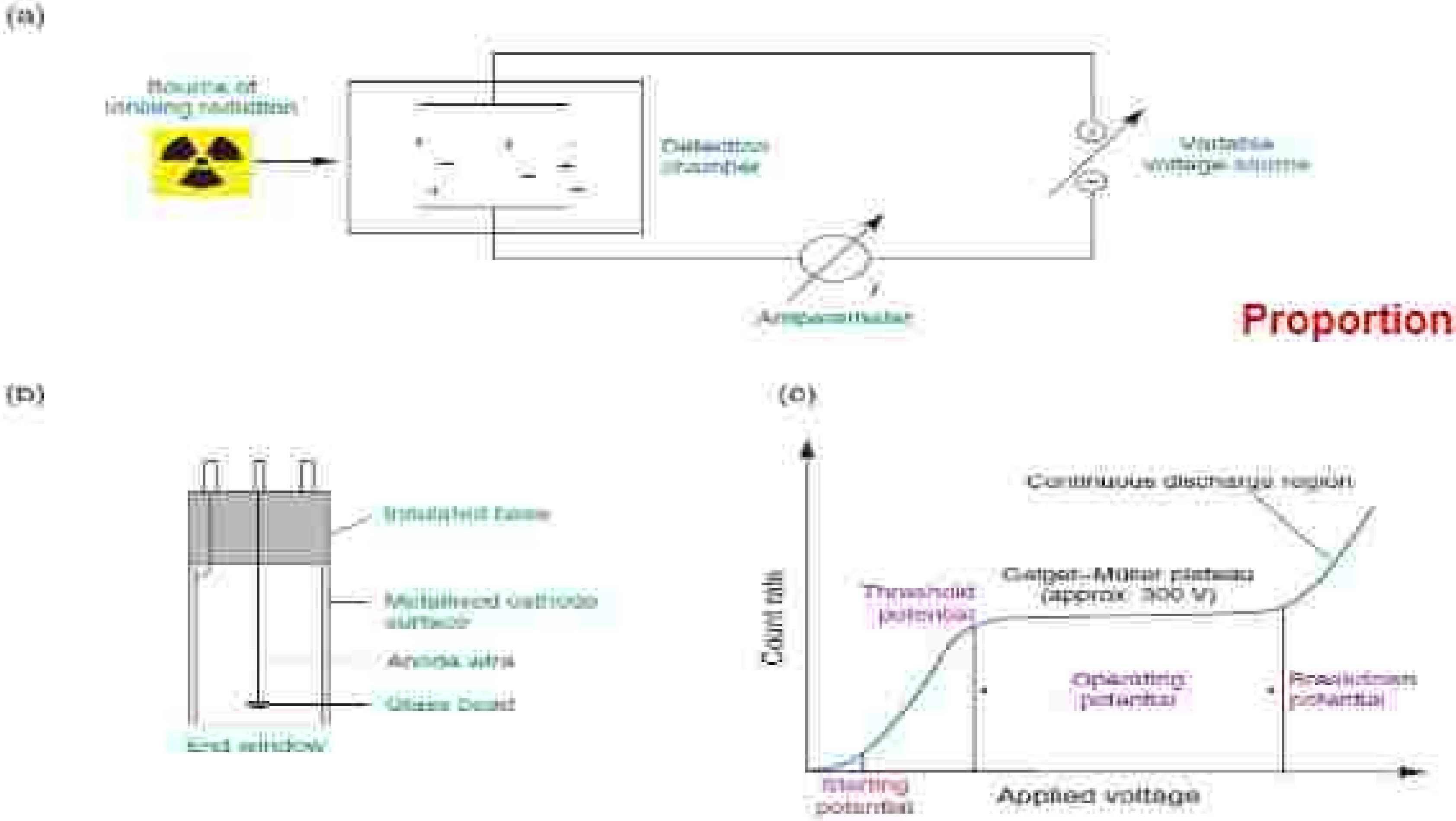
Individual particles such as electrons, neutrons, and protons cannot be seen directly, so their existence must be inferred through measurements. Many different devices, of varying levels of sophistication, have been developed to do this.

MEASUREMENT OF RADIOACTIVITY

Methods Based Upon Gas Ionisation

- If a charged particle passes through a gas, its electrostatic field dislodges electrons from atoms sufficiently close to its path and causes ionisation.
- The ability to induce ionisation decreases in the order
- $\alpha > \beta > \gamma$ with a ratio of about 10 000:100:1.
- In ionisation counters, the ions travel to their respective electrodes; other ionising particles entering the tube during this time (the so-called dead time) are not detected and this reduces the counting efficiency.

MEASUREMENT OF RADIOACTIVITY
Methods Based Upon Gas Ionisation

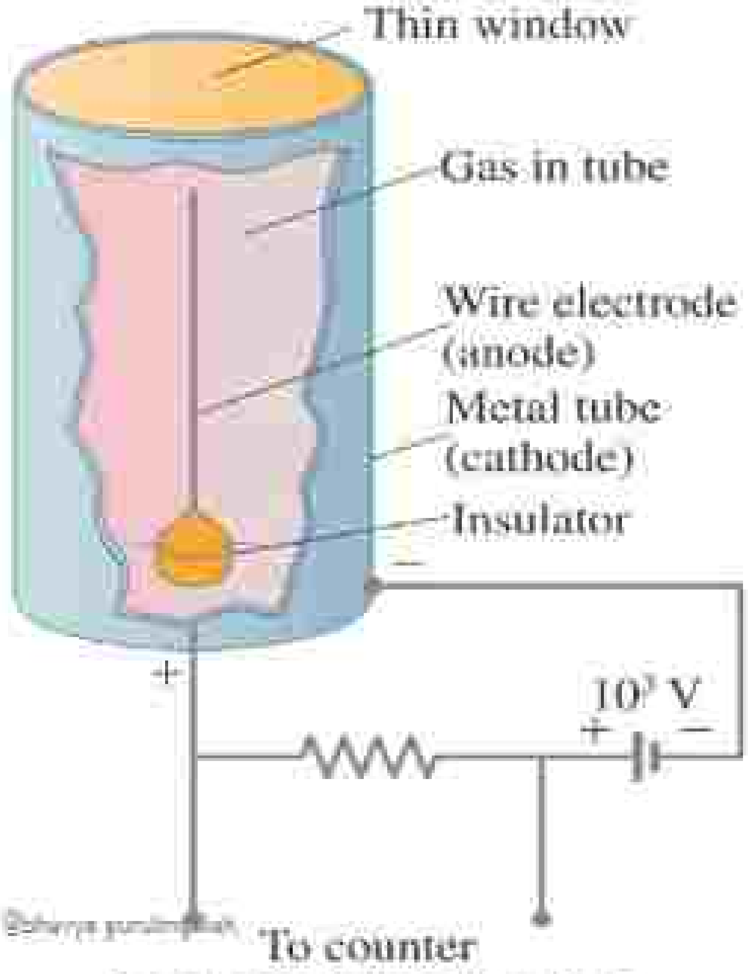


Proportional counters

Figure 9.3 Detection based on ionisation. (a) Schematics of an ionisation counter. (b) The Geiger–Müller tube. (c) The effect of applied voltage on count rate.

MEASUREMENT OF RADIOACTIVITY
Methods Based Upon Gas Ionisation; Geiger Counter

Detection of Radiation



The Geiger counter is a gas-filled tube with a wire in the center. The wire is at high voltage; the case is grounded. When a charged particle passes through, it ionizes the gas. The ions cascade onto the wire, producing a pulse.

The Geiger–Müller counter (Figure 9.3b , c , Figure 9.4a) has a cylindrical- shaped gas chamber and operates at a high voltage, thus eliminating problems with voltage stablity; therefore, the counter is cheaper and lighter than proportional counters

MEASUREMENT OF RADIOACTIVITY
Methods Based Upon Gas Ionisation: Geiger Counter
Importance/Application

- Ionisation counters are used for routine monitoring of the laboratory to check for contamination.
- experimental situations where the presence or absence of radioactivity needs to be known.

b. Methods Based Upon Excitation

scintillation counter

- ionisation and excitation: two ways the radioactive elements interacts with matter.
- Excitation phenomenon with respect to radioactive elements leads to emission of photons and its known as a **scintillation.**
- **Photons are detected using photomultipler, acts as a base from scintillation count.**
- photomultiplier converts the energy of radiation into an electrical signal, and the magnitude of the current that results is directly proportional to the energy of the radiation,
- This means that two, or even more, isotopes can be separately detected and measured in the same sample

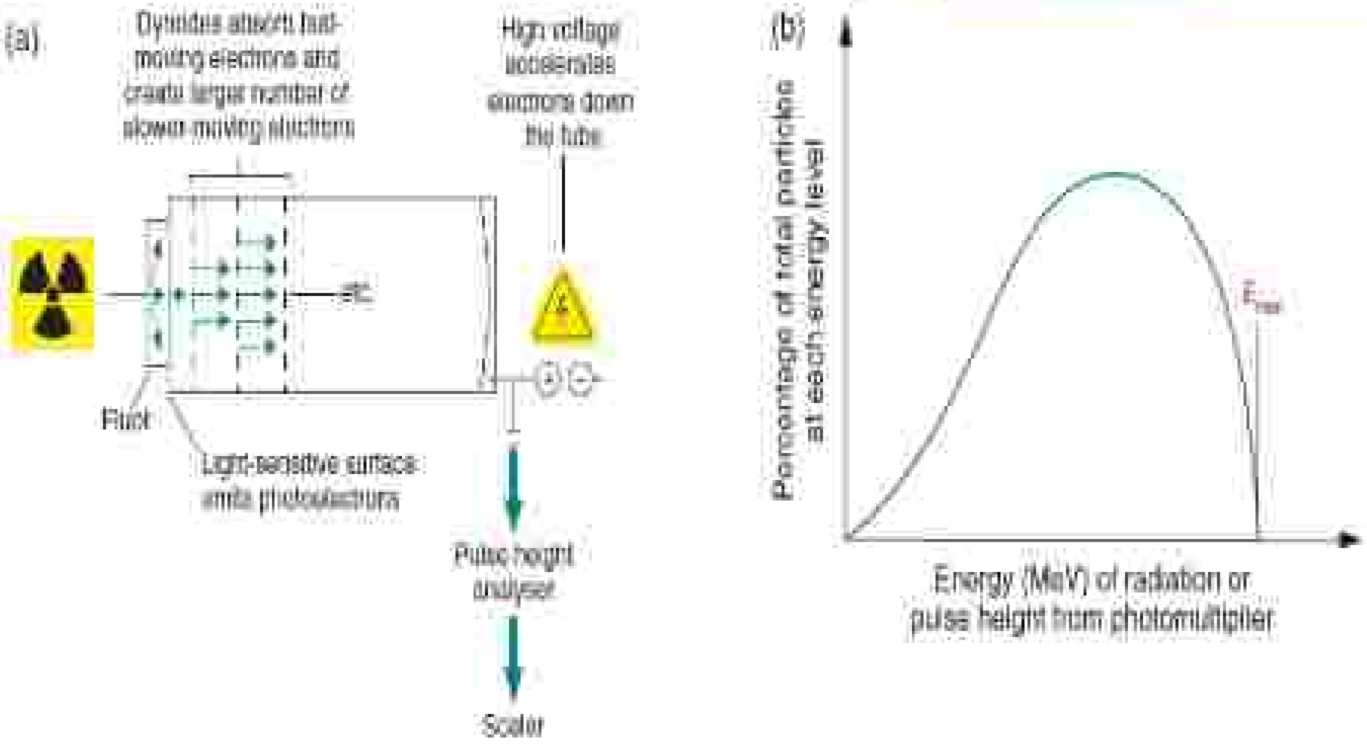
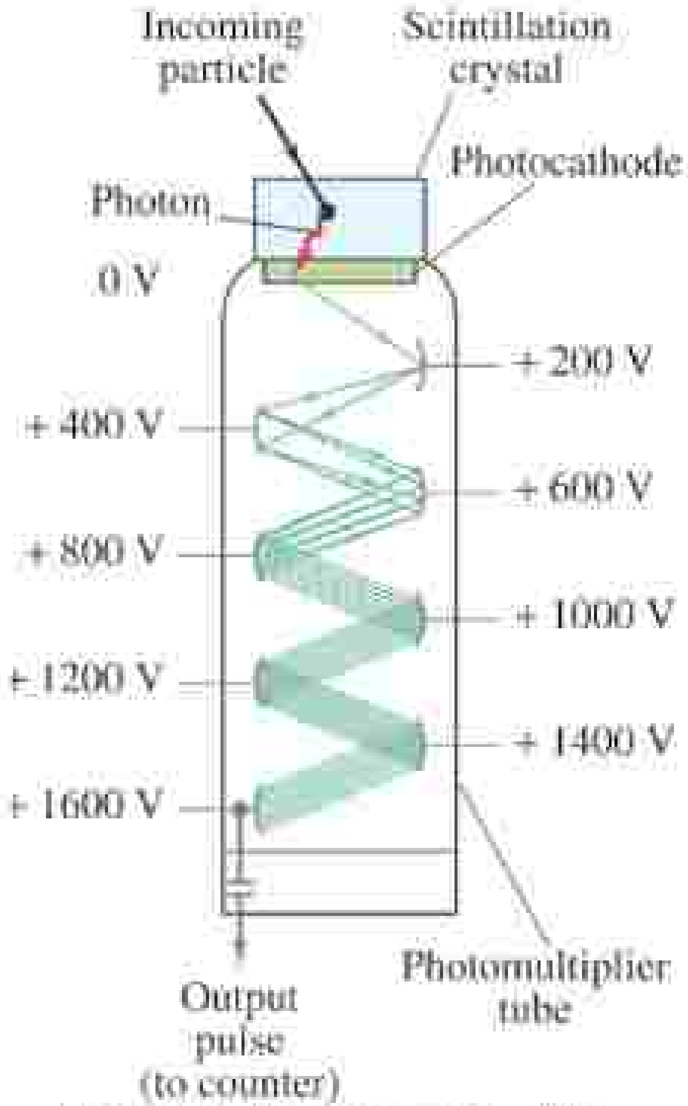


Figure 9.5 (a) The mode of action of a photomultiplier and (b) the energy spectrum of a typical β-emitter.

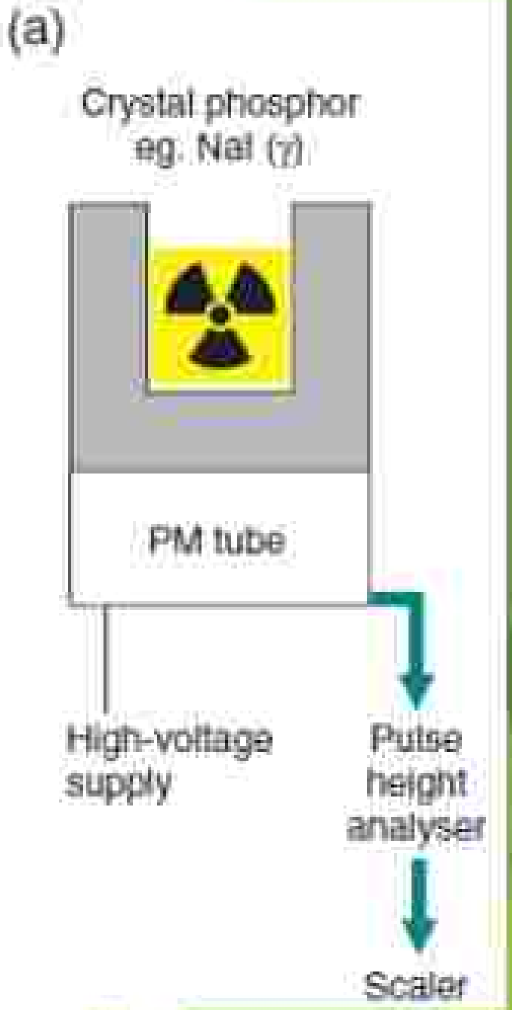
Detection of Radiation



A scintillation counter uses a scintillator – a material that emits light when a charged particle goes through it. The scintillator is made light-tight, and the light flashes are viewed with a photomultiplier tube, which has a photocathode that emits an electron when struck by a photon and then a series of amplifiers.

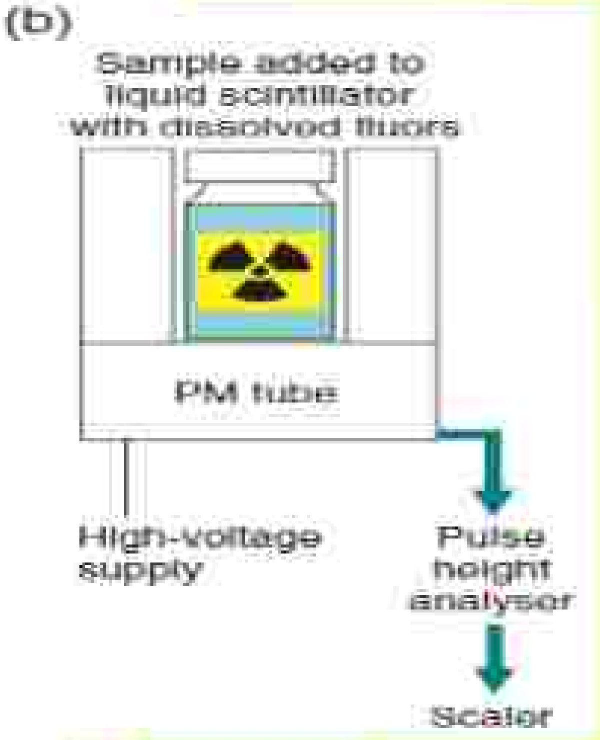
b. Methods Based Upon Excitation
Types of **scintillation counter**

- 2 types
- Solid and Liquid.
- In solid scintillation counting, the sample is placed adjacent to a solid fluor.
- Solid scintillation counting is particularly useful for γ –emitting isotopes, mainly because -rays can penetrate the fluor.
- The counters can be small hand-held devices with the fluor attached to the photomultiplier tube.



b. Methods Based Upon Excitation
Types of **scintillation counter**

- In liquid scintillation counting the sample is mixed with a scintillation fluid containing a solvent and one or more dissolved fluors.
- This method is particularly useful in quantifying weak -emitters such as ^{3}H, ^{14}C and ^{35}S, which are frequently used in biological work.
- Scintillation fluids are called cocktails because there are different formulations, made of a solvents.
- A. such as toluene or diisopropylnaphthalene plus fluors such as 2,5-diphenyloxazole (PPO).
- B. such as toluene or diisopropylnaphthalene plus 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), 2-(4- *t* -butylphenyl)-5-(4-bi-phenyl)-1,3,4-oxydiazole (butyl-PBD)



Advantages and disadvantages of Scintillation Counting

- Very fast so there is effectively no dead time
- Counting efficiencies are high.
- The ability to count samples of many types, including liquids, solids, suspensions and gels.
- The ability to separately count different isotopes in the same sample (used in dual labelling experiments)
- Highly automated

Disadvantages

- Cost of the instrument and cost per sample is high.
- Quenching, phospholuminescence, and chemiluminescence.

Determination of counting efficiency

One way to do this is to use an internal standard called a spike. The sample is counted and gives a reading of A counts per minute (c.p.m.). Then, a small amount of standard material of known disintegrations per minute with B d.p.m. is added. The sample is measured again, yielding C c.p.m. This allows calculation of the counting efficiency of the sample:

$$\text{Counting efficiency} = \frac{C - A}{B} \times 100\% \qquad \text{(Eq 9.13)}$$

Cerenkov radiation

- The Cerenkov effect occurs when a particle passes through a substance with a speed higher than that of light passing through the same substance.
- If a β-emitter has a decay energy value in excess of 0.5MeV, then this causes water to emit a bluish white light usually referred to as Cerenkov light/ radation.
- Detection by conventional liquid scintillation counter.
- No requirement for organic solvents and fluors, this technique is relatively cheap, sample preparation is very easy and there is no problem with chemical quenching.

C. Methods Based Upon Exposure of Photographic Emulsions
Autobiography

- Autoradiography - Ionising radiation acts upon a photographic emulsion or film to produce a latent image very much like visible light.
- silver halide crystals
- As energy from the radioactive material is dissipated, the silver halide becomes *negatively charged and is reduced to elementary silver, thus forming a particulate latent image.*
- A very sensitive technique and has been used in a wide variety of biological experiments.
- An autoradiography emulsion or X-ray film is placed as close as possible to the sample and exposed at any convenient temperature.
- Quantitative images are produced until saturation is reached.
- The shades of grey in the image are related to a combination of levels of radiation and length of exposure, until a black image results. Isotopes with an energy of radiation equal to, or higher than, carbon-14 (E max = 0.156MeV) are required.
- The higher the energy the quicker the results will be obtained.
- *Weak -emitting isotopes (e.g. tritium, carbon-14 and sulfur-35) are most suitable for autoradiography, particularly for cell and tissue localisation experiments.*
- *Emitters with higher energy (e.g. phosphorus-32) yield faster results, but poorer resolution.*

C. Methods Based Upon Exposure of Photographic Emulsions Autobiography

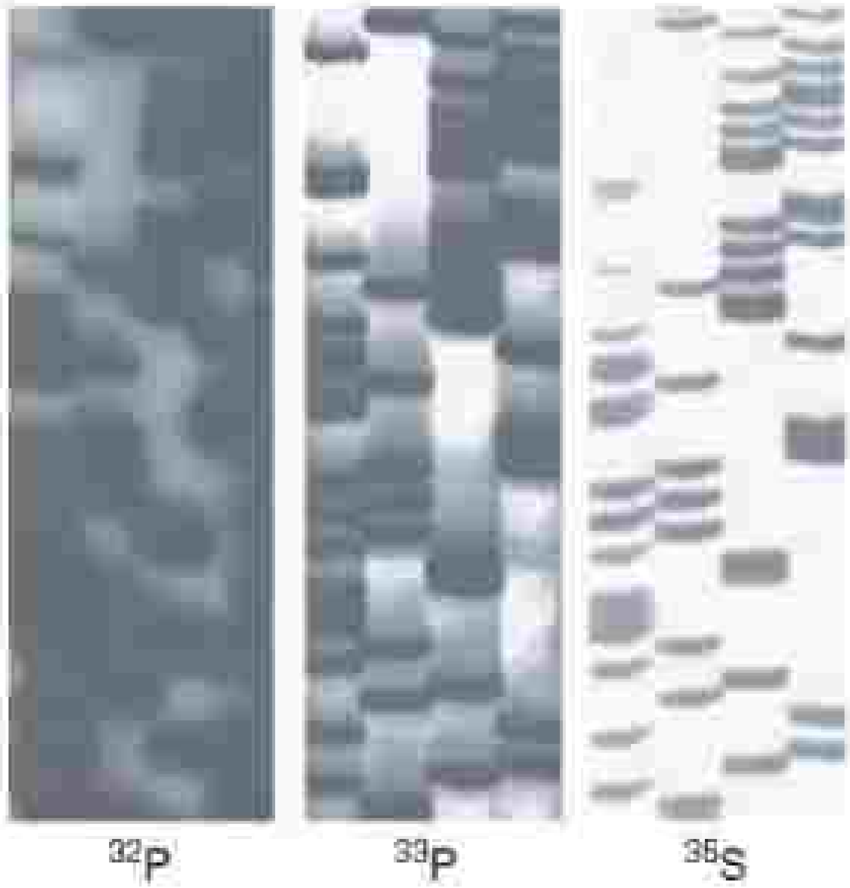
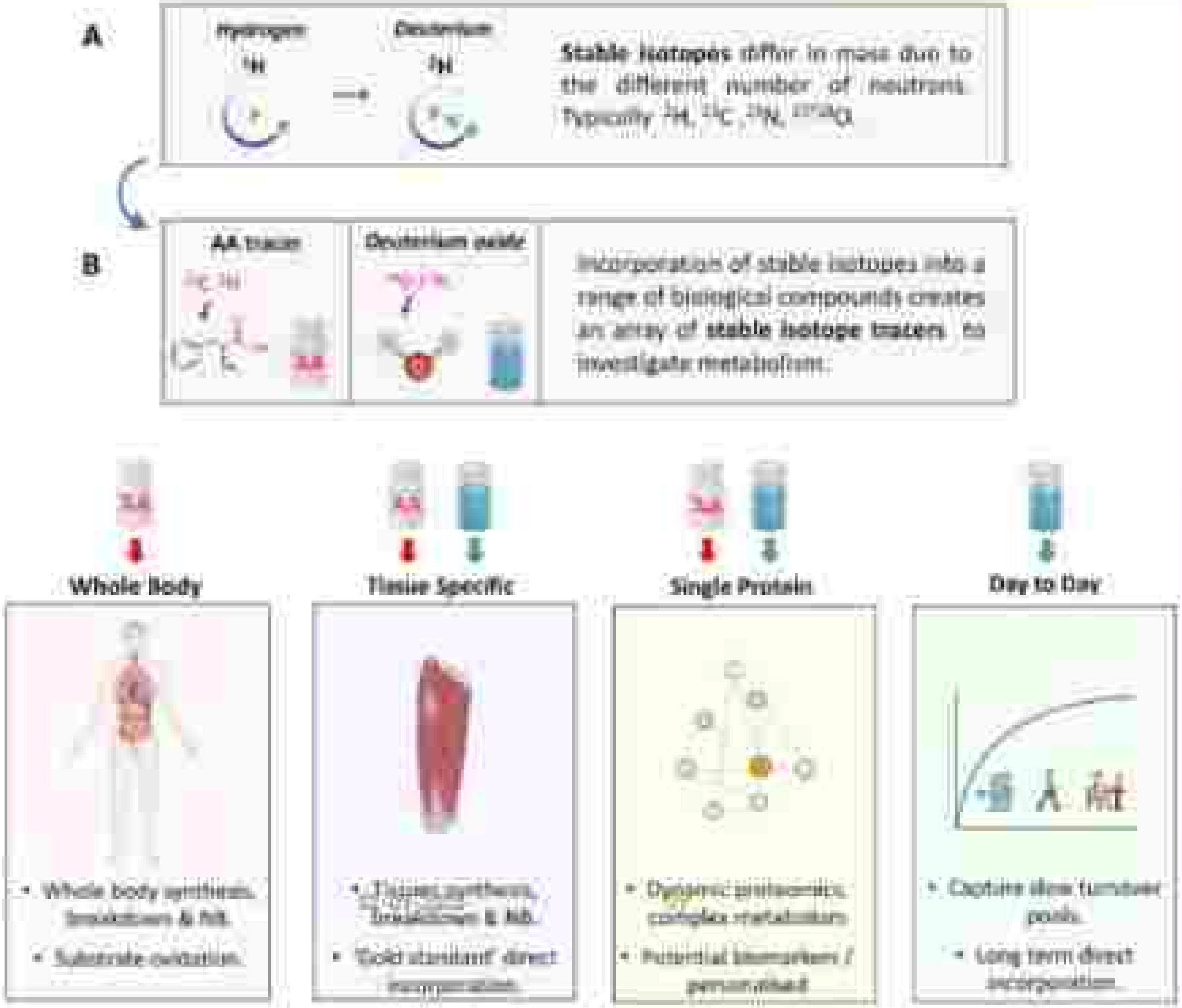


Figure 9.10 Three autoradiographs showing the use of different radioisotopes in DNA sequencing. The isotope with the highest energy (phosphorus-32) leads to the poorest resolution, because the radiation spreads out further, making the DNA bands appear thicker. The lowest-energy radiation (from sulfur-35) yields images with the best resolution. (Reproduced with permission from Cunningham M.W., Patel A., Simmonds A.C. and Williams D (2002) In vitro labelling of nucleic acids and proteins. In *Radioisotopes in Biology*, 2nd Edn., ed. Slater R.J., Oxford University Press, Oxford, UK.)

Stable isotopes – measurement and applications

- Stable isotopes are non-radioactive forms of atoms.
- Do not emit radiation
- Broad variety of applications, including water and soil management, environmental studies, nutrition assessment studies and forensics.
- Eighty out of the first 82 elements in the periodic table have stable isotopes.
- Deuterium (^{2}H) : nutrition research,
- Nitrogen (^{15}N): agriculture
- Stable isotopes include oxygen, carbon, nitrogen, hydrogen and sulfur.

Bhavya gundlingesh



Stable isotopes differ in mass due to the different number of neutrons. Typically ^{2}H, ^{13}C, ^{15}N, $^{17/18}$O.

Incorporation of stable isotopes into a range of biological compounds creates an array of **stable isotope tracers** to investigate metabolism.

- Whole body synthesis, breakdown & NB.
- Substrate oxidation.

- Tissue synthesis, breakdown & NB.
- 'Gold standard' direct incorporation.

- Dynamic proteomics, complex metabolism
- Potential biomarkers / personalised

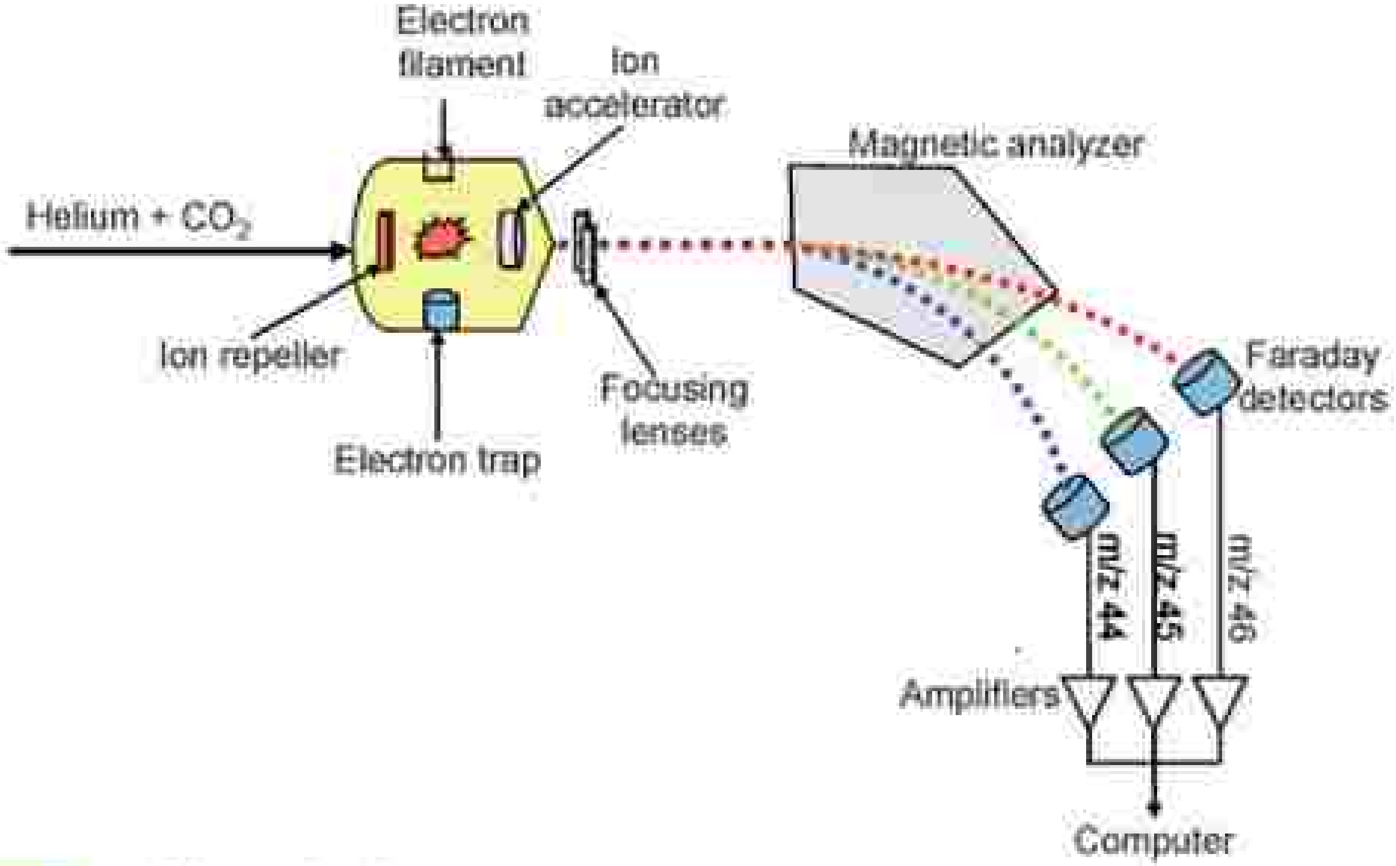
- Capture slow turnover pools.
- Long term direct incorporation.

Stable isotopes – measurement and applications Isotope Ratio Mass Spectrometry (IRMS)

- Specialized technique.
- geographic, chemical, and biological origins of substance
- The ability to accurately and precisely measure variations in the abundance of isotopic ratios of light elements such $^{13}C/^{12}C$, $^{18}O/^{16}O$, $D/^{1}H$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$.
- Isotope ratios of samples of interest are measured relative to universal standards and are reported in the delta notation, d:

$$\delta = 1000(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}$$

Stable isotopes – measurement and applications Isotope Ratio Mass Spectrometry (IRMS)



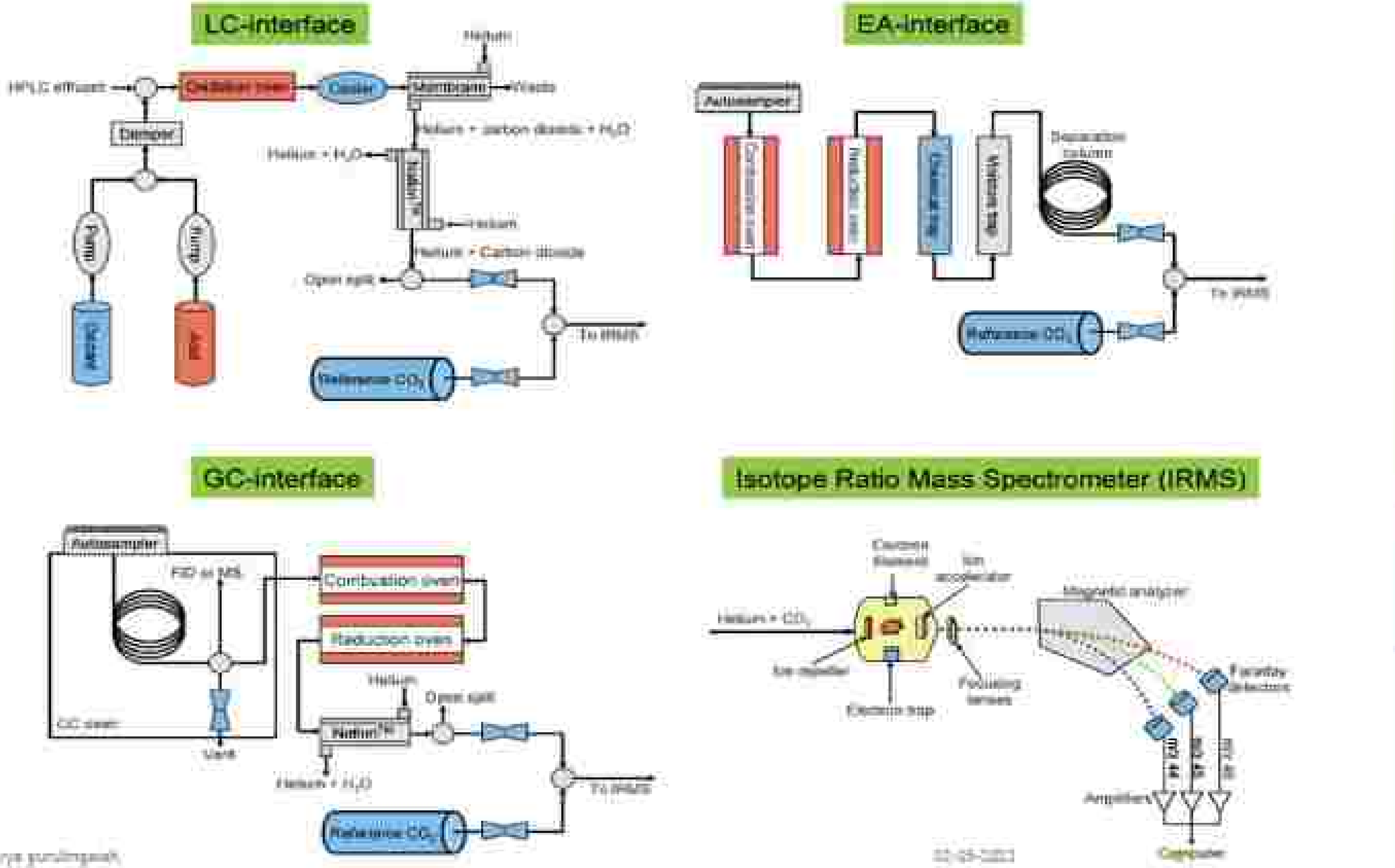
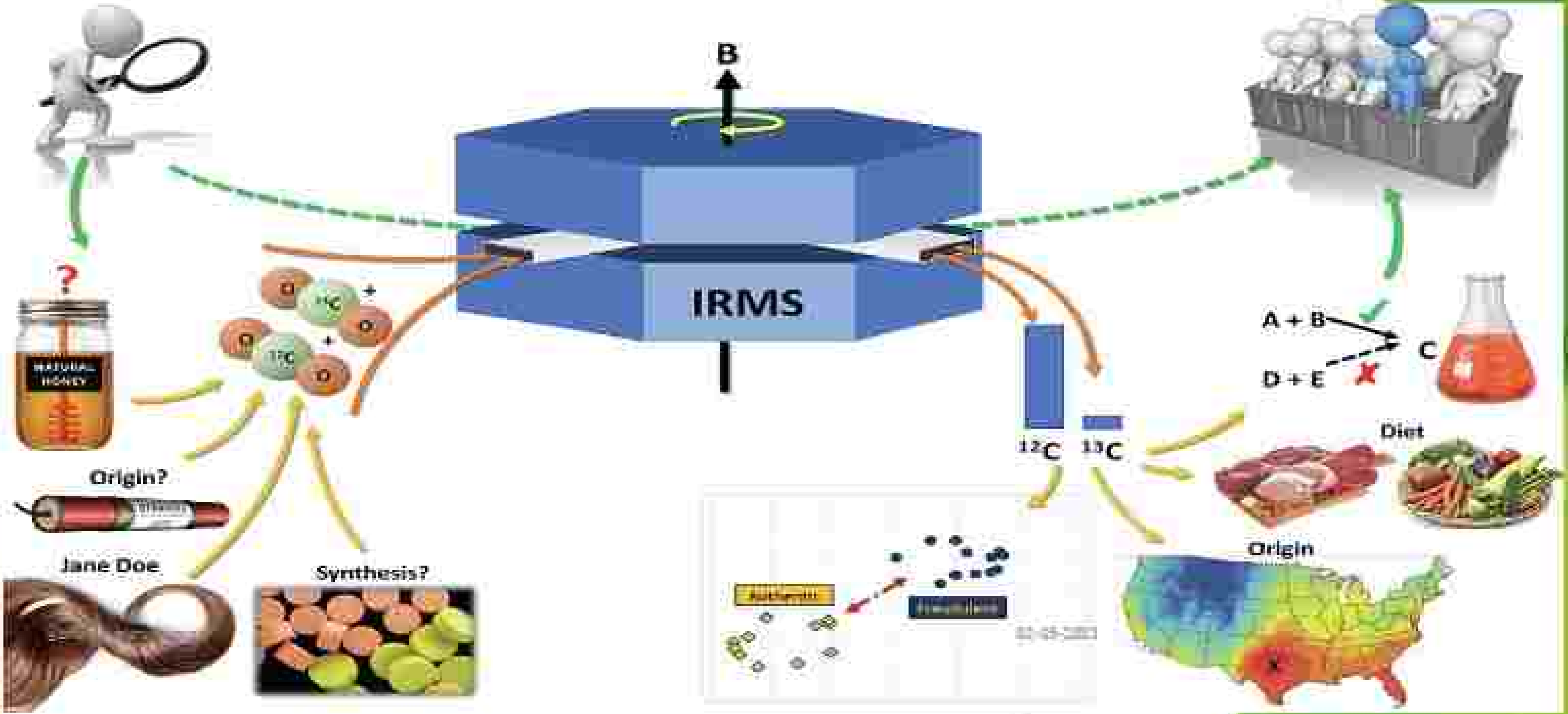
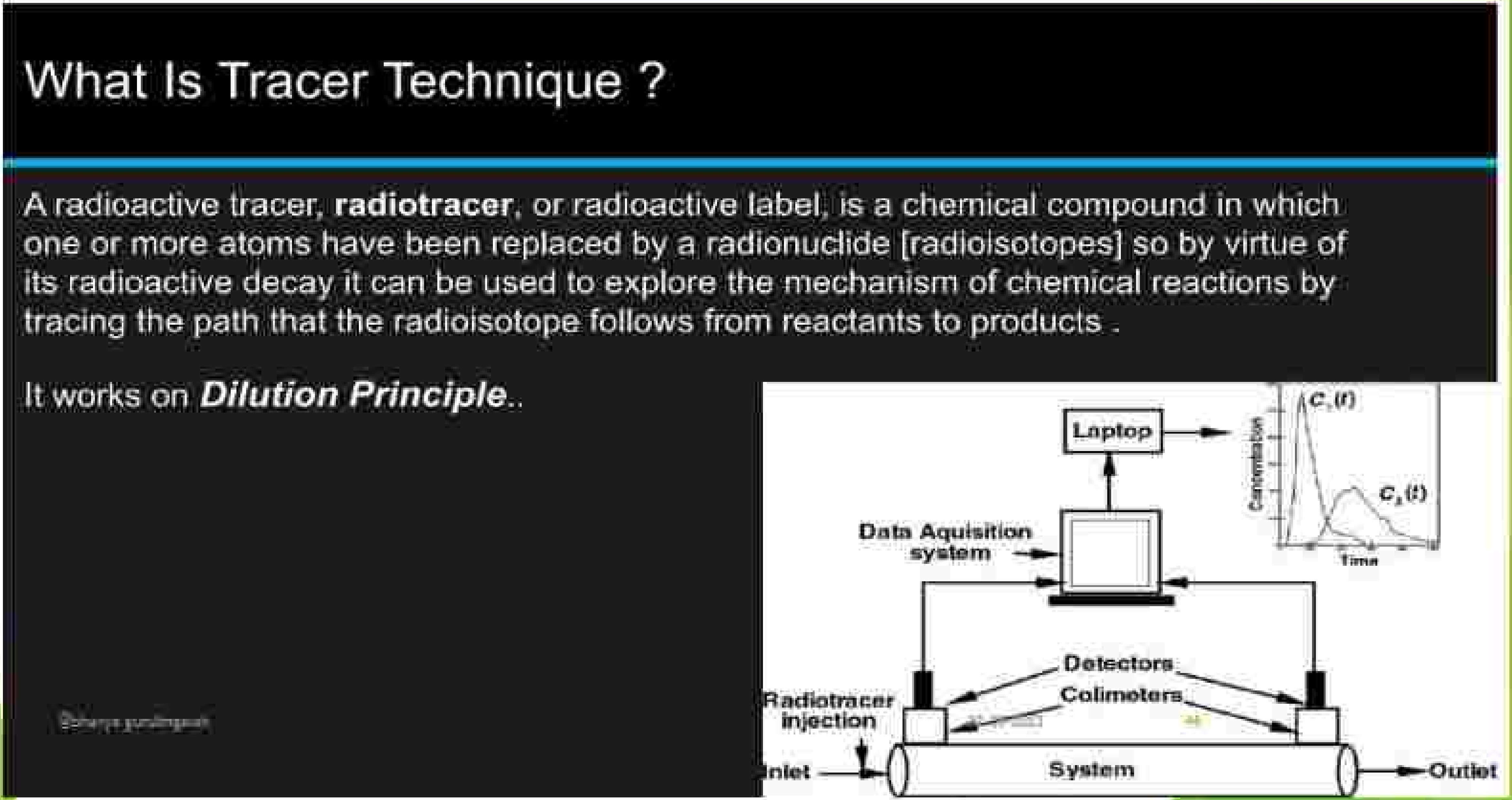


Fig. 2 Schematics to show how the three most common sample introduction systems/interfaces for carbon isotope measurements (as CO_2) and an isotope ratio mass spectrometer. LC = liquid chromatography, EA = elemental analyzer, GC = gas chromatography.

Stable isotopes – measurement and applications
Isotope Ratio Mass Spectrometry (IRMS)



Radiotracer Techniques

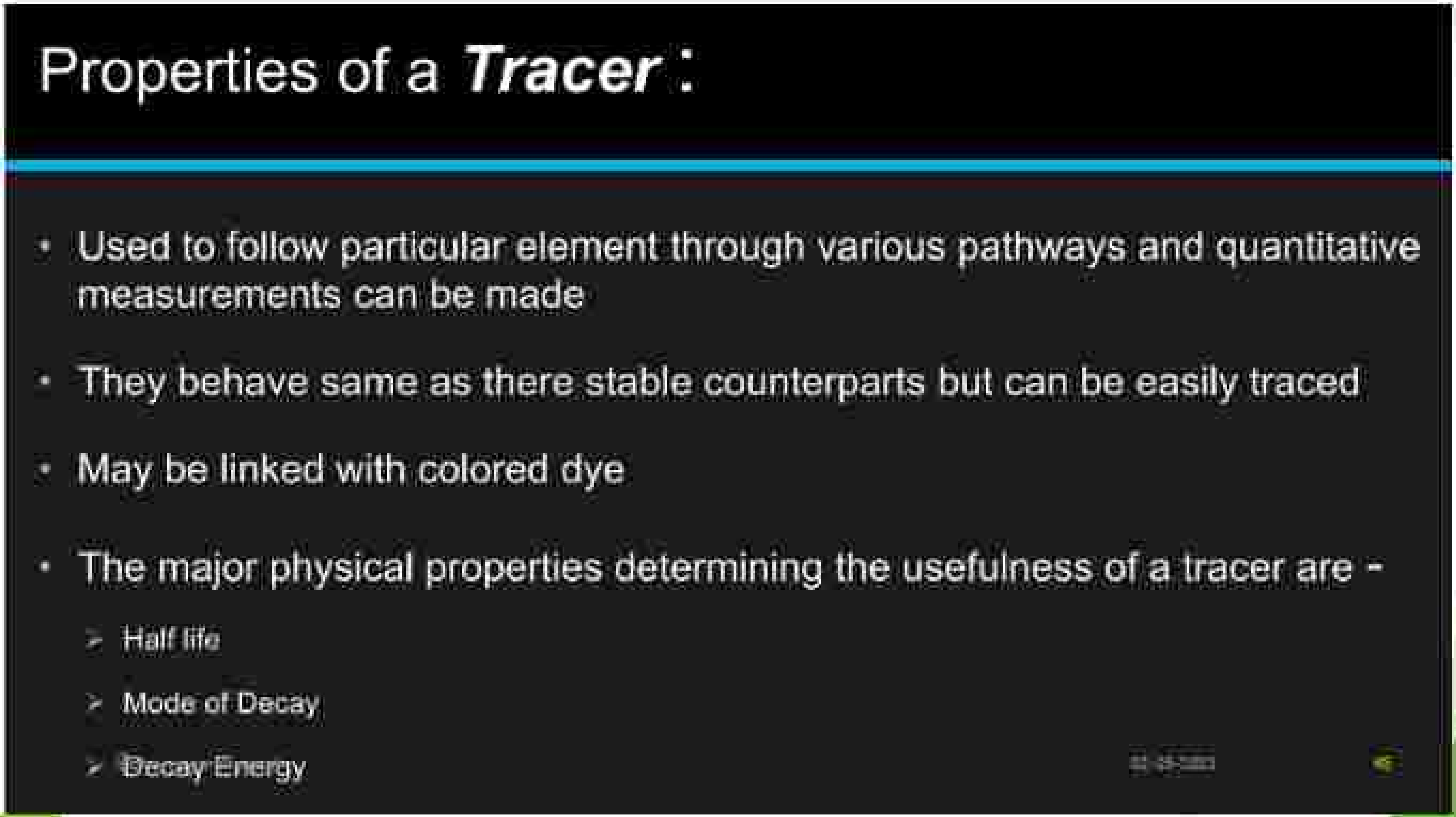


What Is Tracer Technique ?

A radioactive tracer, **radiotracer**, or radioactive label, is a chemical compound in which one or more atoms have been replaced by a radionuclide [radioisotopes] so by virtue of its radioactive decay it can be used to explore the mechanism of chemical reactions by tracing the path that the radioisotope follows from reactants to products .

It works on ***Dilution Principle***..

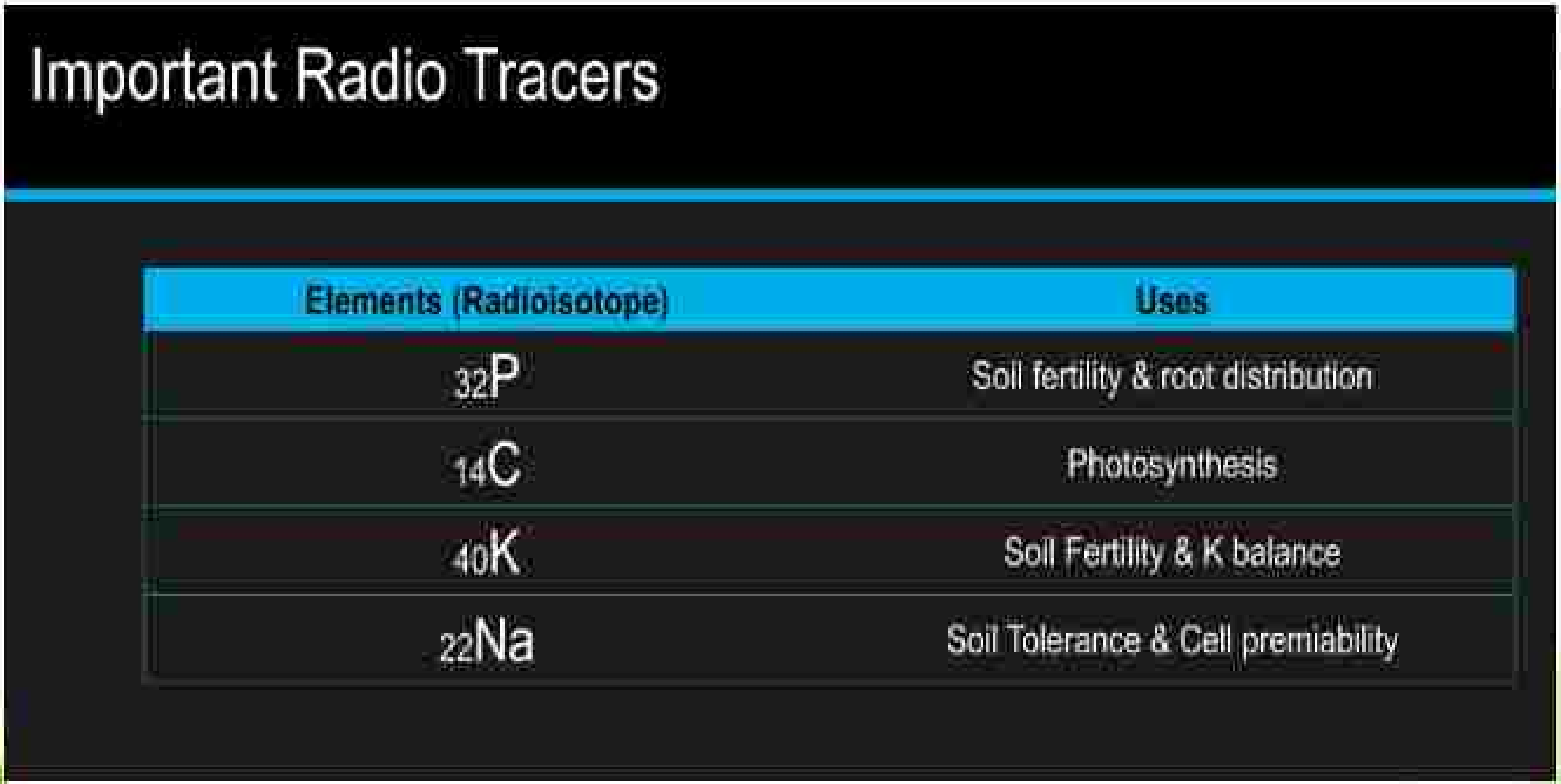
Radiotracer Techniques



Properties of a ***Tracer*** :

- Used to follow particular element through various pathways and quantitative measurements can be made
- They behave same as there stable counterparts but can be easily traced
- May be linked with colored dye
- The major physical properties determining the usefulness of a tracer are -
  - Half life
  - Mode of Decay
  - Decay Energy

Radiotracer Techniques



Important Radio Tracers

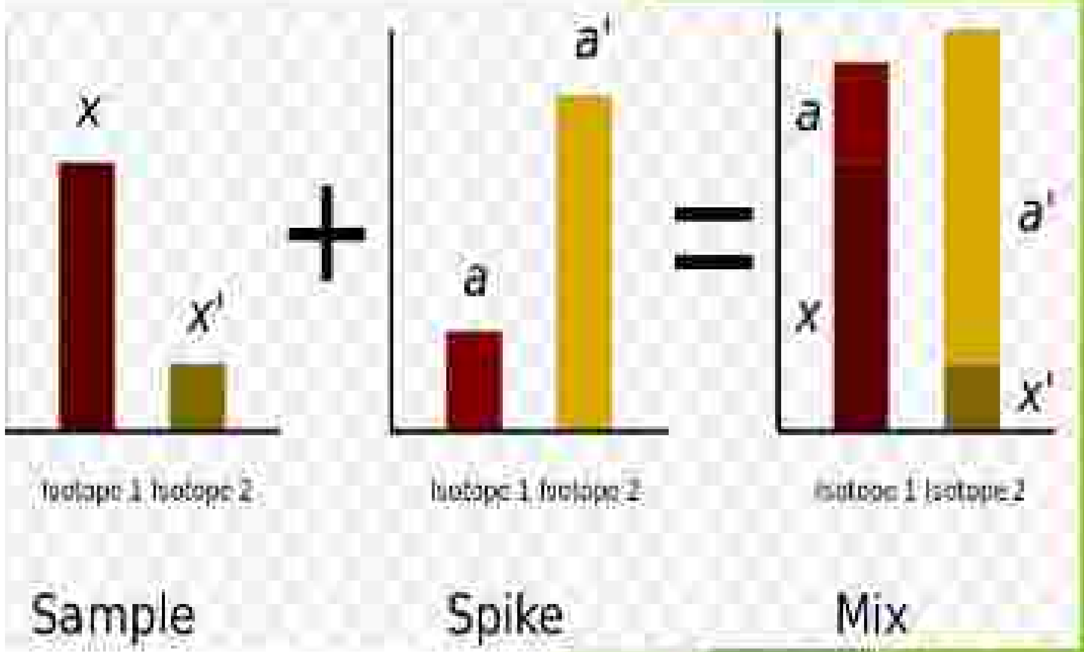
Elements (Radioisotope)	Uses
$_{32}P$	Soil fertility & root distribution
$_{14}C$	Photosynthesis
$_{40}K$	Soil Fertility & K balance
$_{22}Na$	Soil Tolerance & Cell premiability

Radiotracer Techniques

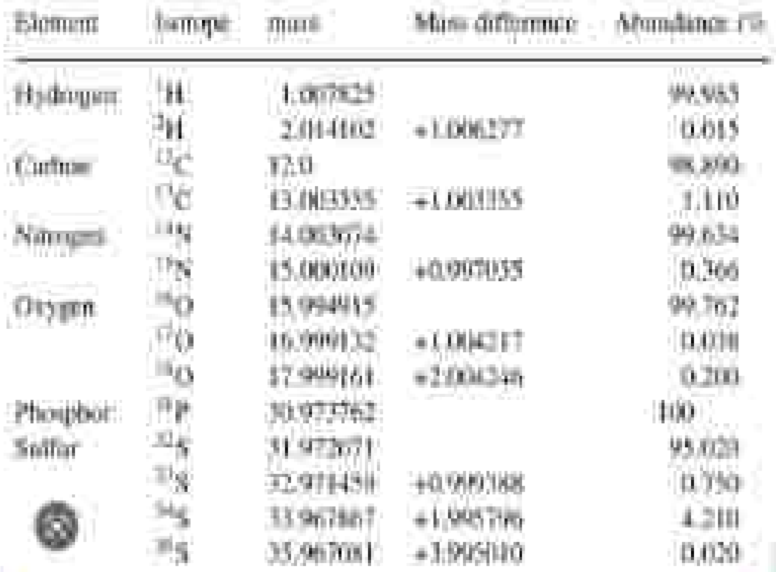
- The principle of a tracer study is that *it allows the analysis of a substance and its interactions in the body through the labeling of the substance with a radionuclide in a manner that does not alter the substances original properties.*
- The main steps in a tracer study include *selection and administration of the radiotracer, sampling, sample preparation and measurement.*
- The most commonly used equipment for sample measurements include the gamma-counter and the liquid scintillation counter.
- Also external probes (e.g. for thyroid uptake measurement, surgical probes for detection of sentinel node),
- the gamma-camera (bile malabsorption) and
- the PET-camera (biomedical research in general) are used.

Isotope dilution

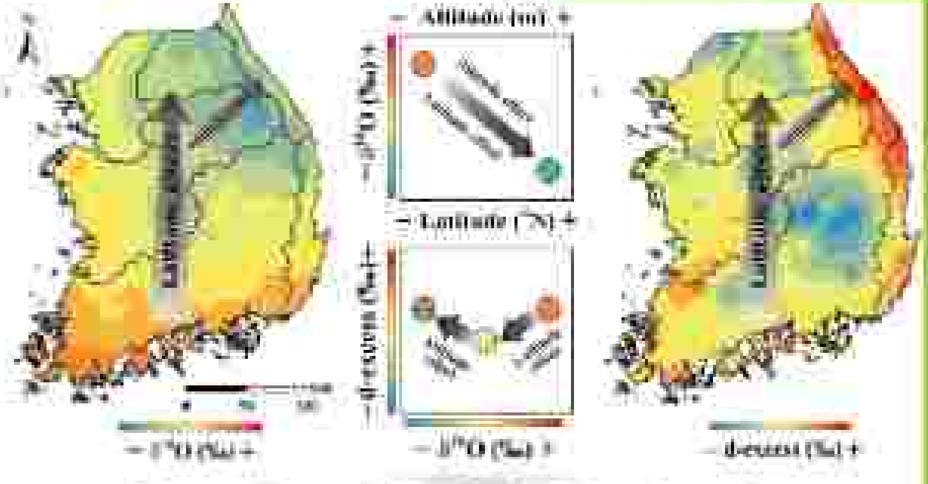
- **Isotope dilution**, radiochemical method of analysis for measuring the mass and quantity of an element in a substance.
- The procedure involves adding to a substance a known quantity of a radioisotope of the element to be measured and mixing it with the stable isotope of the element.
- A sample is then taken from the mixture and analyzed.
- By measuring the amount of radioactive isotope and the amount of stable isotope present and determining the ratio of these amounts, both the quantity and mass of the element can be ascertained.



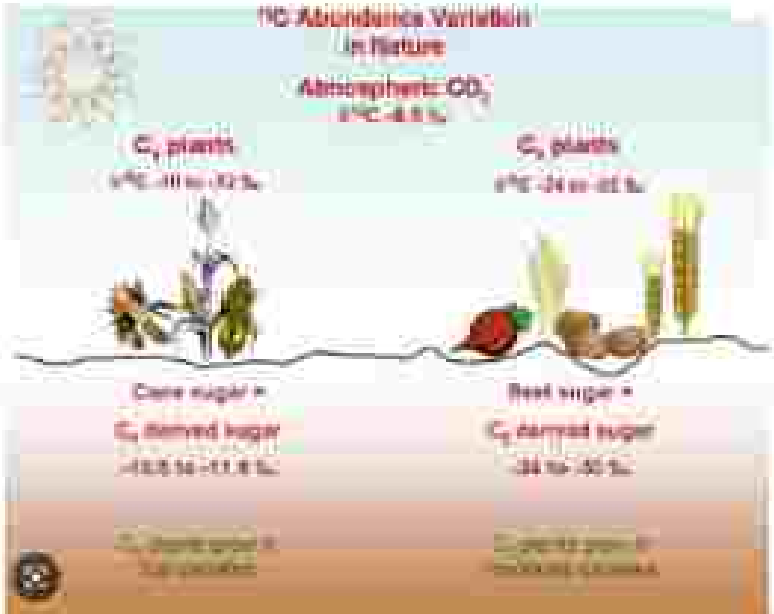
Applications: Biochemistry, distribution studies, metabolite studies, radio immuno assay for detection of Ag/Ab



Element	Isotope	mass	Mass difference	Abundance (%)
Hydrogen	^{1}H	1.007825		99.985
	^{2}H	2.014102	+1.006277	0.015
Carbon	^{12}C	12.0		98.890
	^{13}C	13.003355	+1.003355	1.110
Nitrogen	^{14}N	14.003074		99.634
	^{15}N	15.000109	+0.997035	0.366
Oxygen	^{16}O	15.994915		99.762
	^{17}O	16.999132	+1.004217	0.038
	^{18}O	17.999161	+2.004246	0.200
Phosphor	^{31}P	30.973762		100
Sulfur	^{32}S	31.972071		95.020
	^{33}S	32.971459	+0.999388	0.750
	^{34}S	33.967867	+1.995796	4.210
	^{36}S	35.967081	+3.995010	0.020



Understanding spatial distribution of groundwater isotope and its controlling factors on a national scale

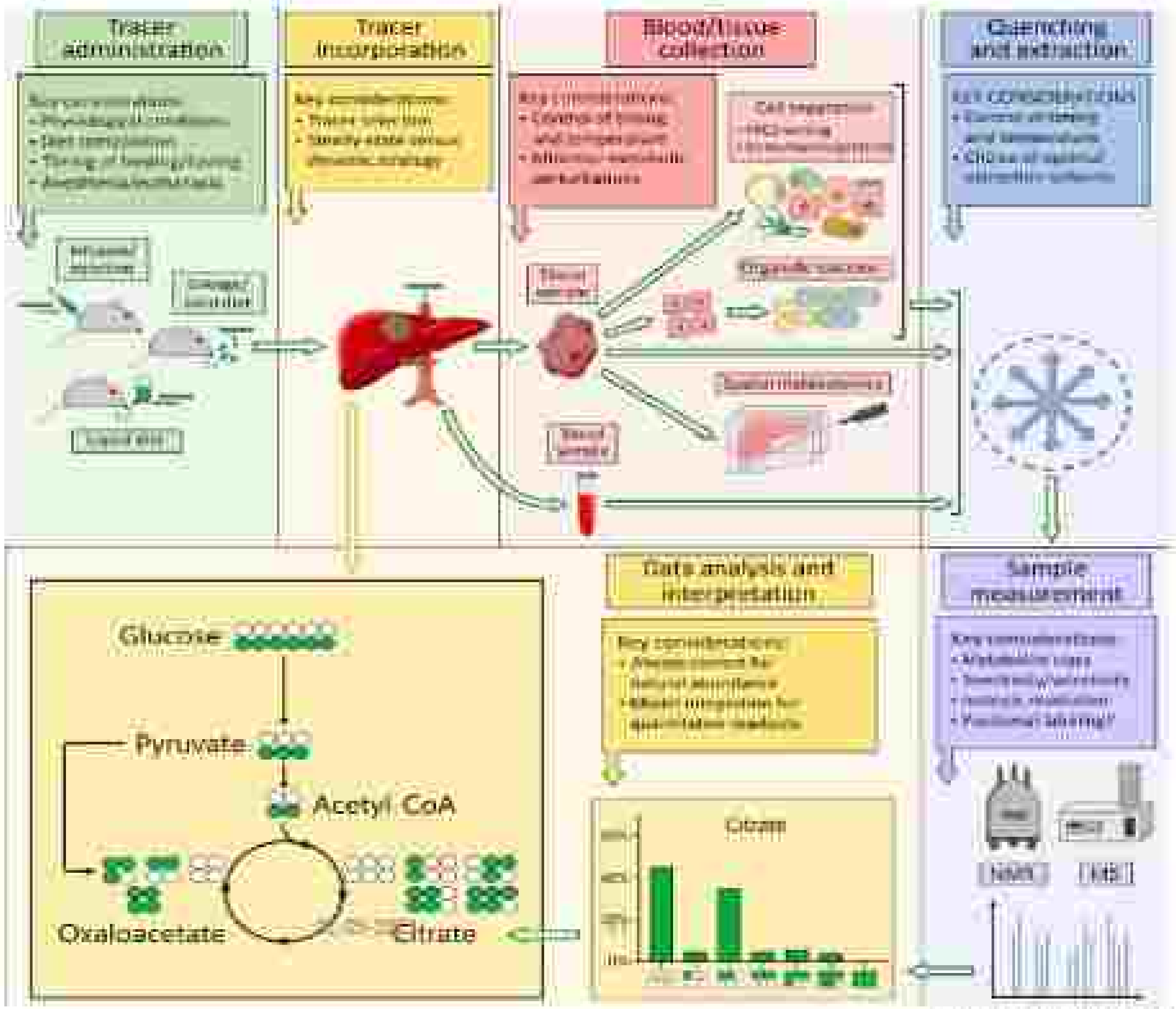


Applications: Biochemistry, distribution studies, metabolite studies, radio immuno assay for detection of Ag/Ab

Applications: Biochemistry, distribution studies, metabolite studies, clinical applications, radio immuno assay for detection of Ag/Ab

Outline of the Different Steps Involved in an In Vivo Stable-Isotope Tracing Experiment. Appropriate stable-isotope tracers chosen based on the biological question of interest are administered to the model organism using various approaches such as infusions/injections, gavage, or solid/liquid diets. Tissue or blood samples are later collected from the subject and processed according to the readout of interest. For tissue samples, this may include: (i) whole-tissue quenching, (ii) quenching following cell and/or organelle isolation, or (iii) preparation for spatial metabolomics analysis. Metabolites are then extracted to measure the incorporation of isotopic label from the tracer into downstream metabolic products, using analytical techniques such as mass spectrometry (MS) or NMR. The isotopic enrichment profiles of different metabolites can then be assessed and interpreted to gain insight on the activities of different intracellular metabolic pathways. Alternatively, these enrichment profiles can be combined with extracellular (blood) measurements and integrated into systems-level metabolic models, to achieve quantitative readouts of the subject's metabolic state

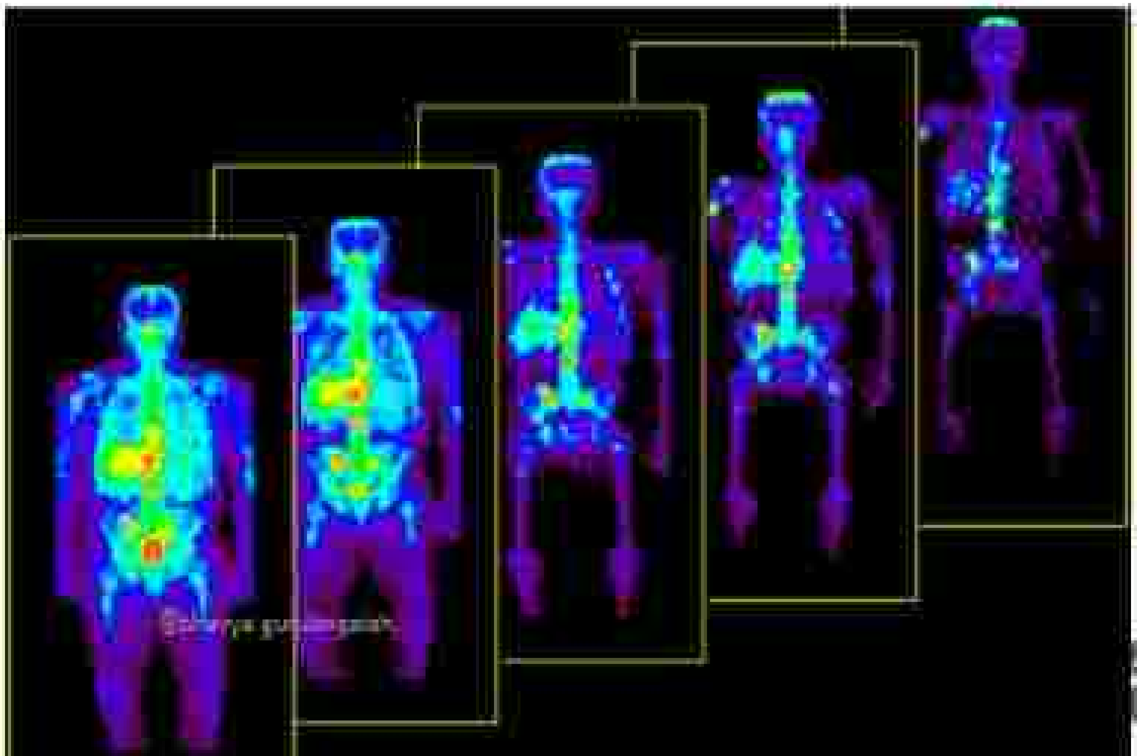
Refer: Juan Fernández-García et al., 2019



Trends in Biochemical Sciences

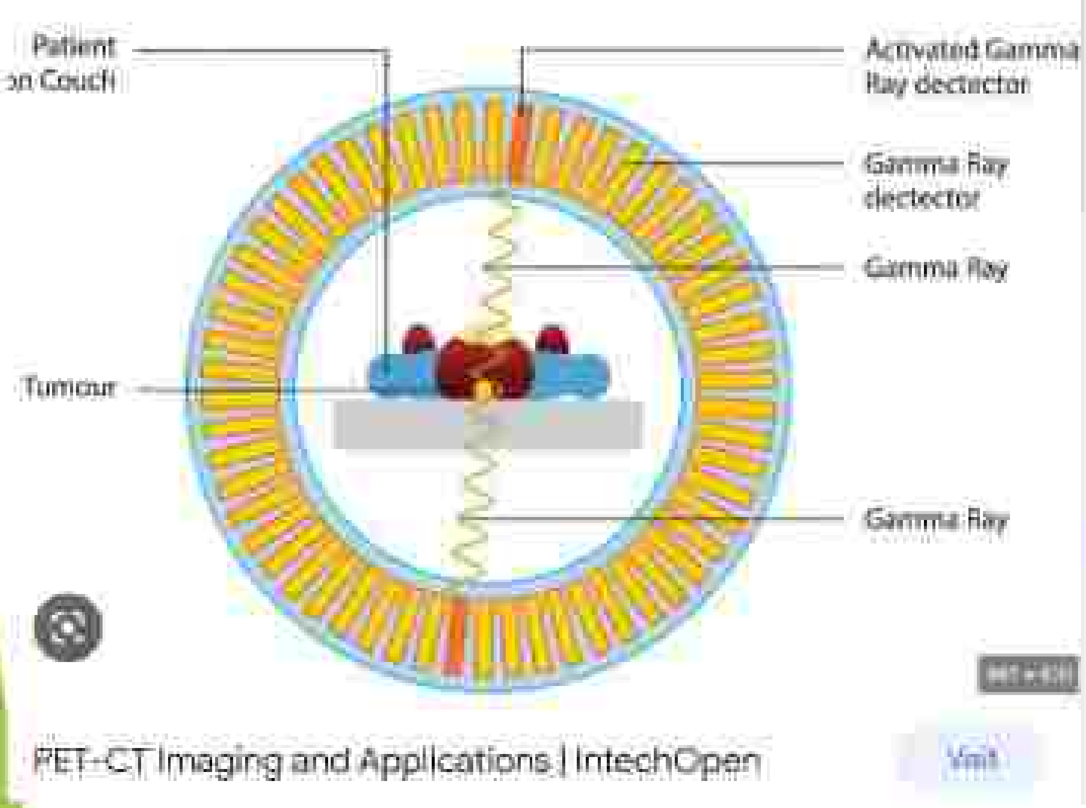
Applications: Biochemistry, distribution studies, metabolite studies, clinical applications, radio immuno assay for detection of Ag/Ab

- The radiation therapy in oncology, where both correct diagnose imaging and proper treatment technique are at high stakes.
- Radiotherapy use radiation to weaken or destroy particular targeted cells with low toxic side-effects.
- Radioisotopes in combination with imaging devices study the processes of the various parts of the body.
- Method allows to Conduct functional imaging of the nuclide distribution, both static and dynamic
- radioactive trackers are short-lived isotopes linked to chemical compounds which permit specific body processes to be tested.
- Radionuclides for nuclear medicine are produced at nuclear reactors, nuclear accelerators, and radionuclide generators

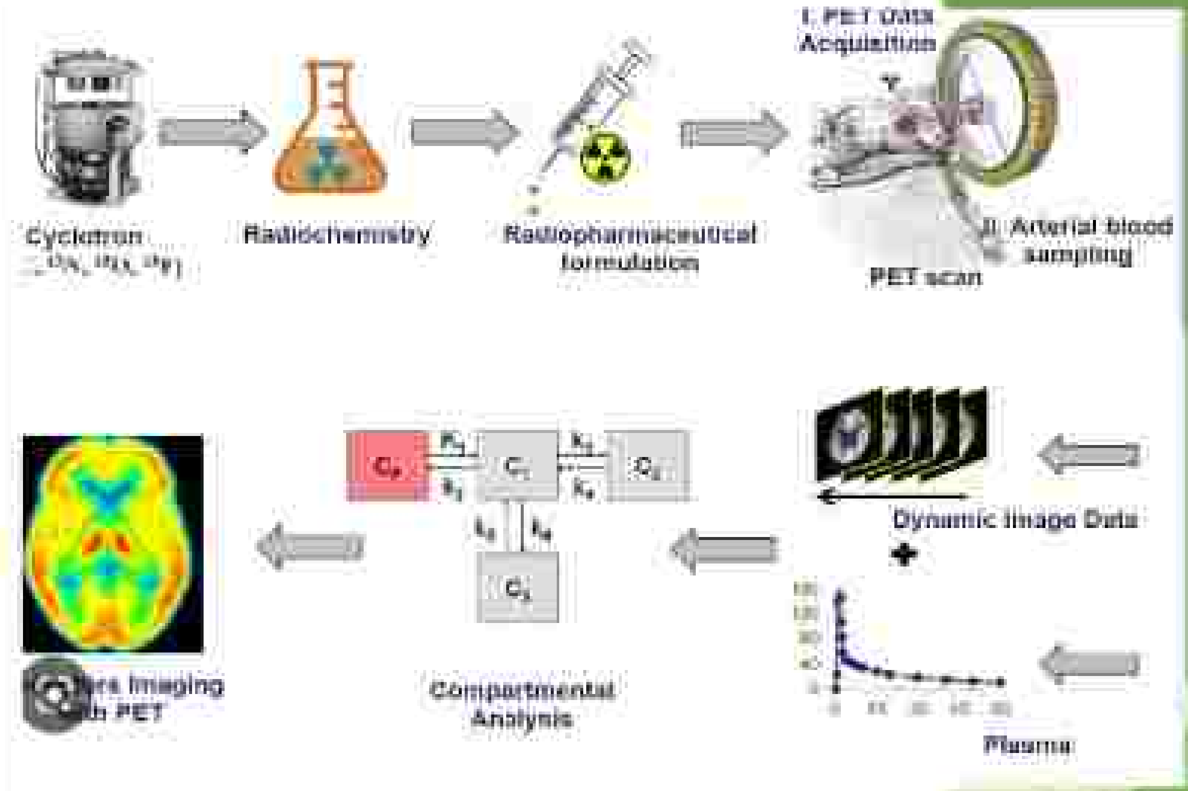


Radioactive Isotope	Applications in Medicine
Cobalt-60	Radiation therapy to prevent cancer
Iodine-131	Locate brain tumors, monitor cardiac, liver and thyroid activity
Carbon-14	Study metabolism changes for patients with diabetes, gout and anemia
Carbon-11	Tagged onto glucose to monitor organs during 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)

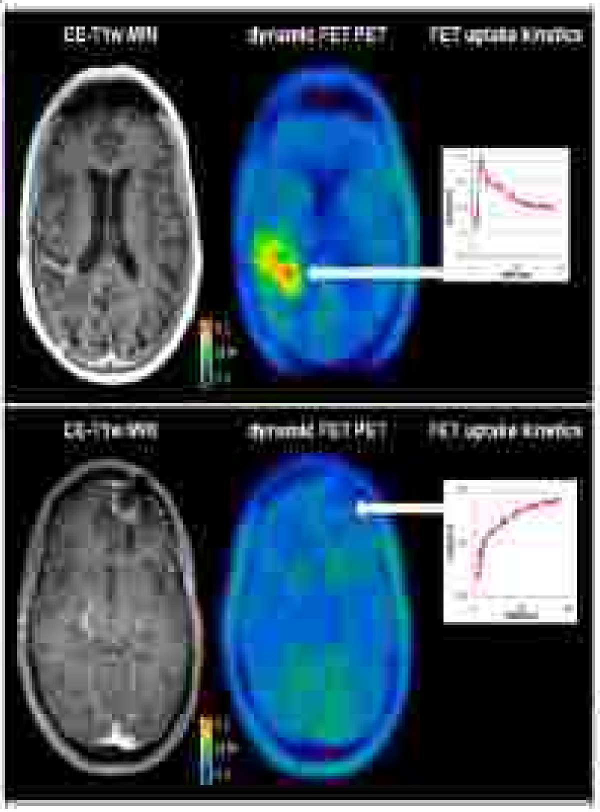
Applications: Biochemistry, distribution studies, metabolite studies, clinical applications, radio immuno assay for detection of Ag/Ab

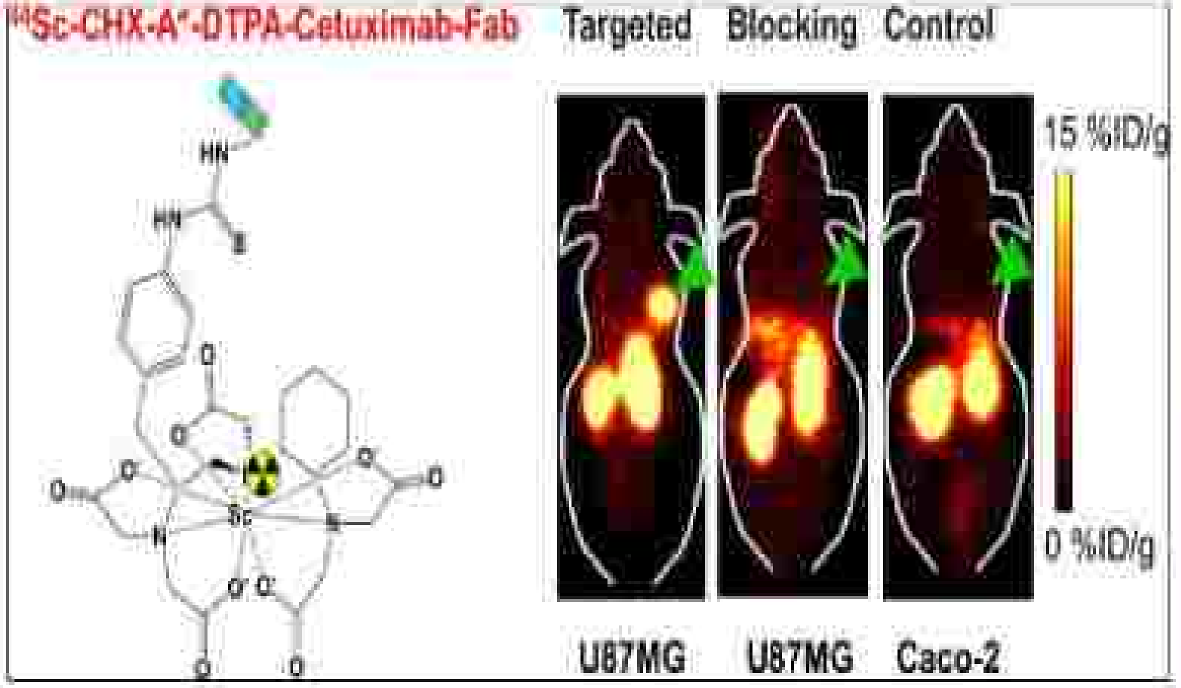


PET-CT Imaging and Applications | IntechOpen



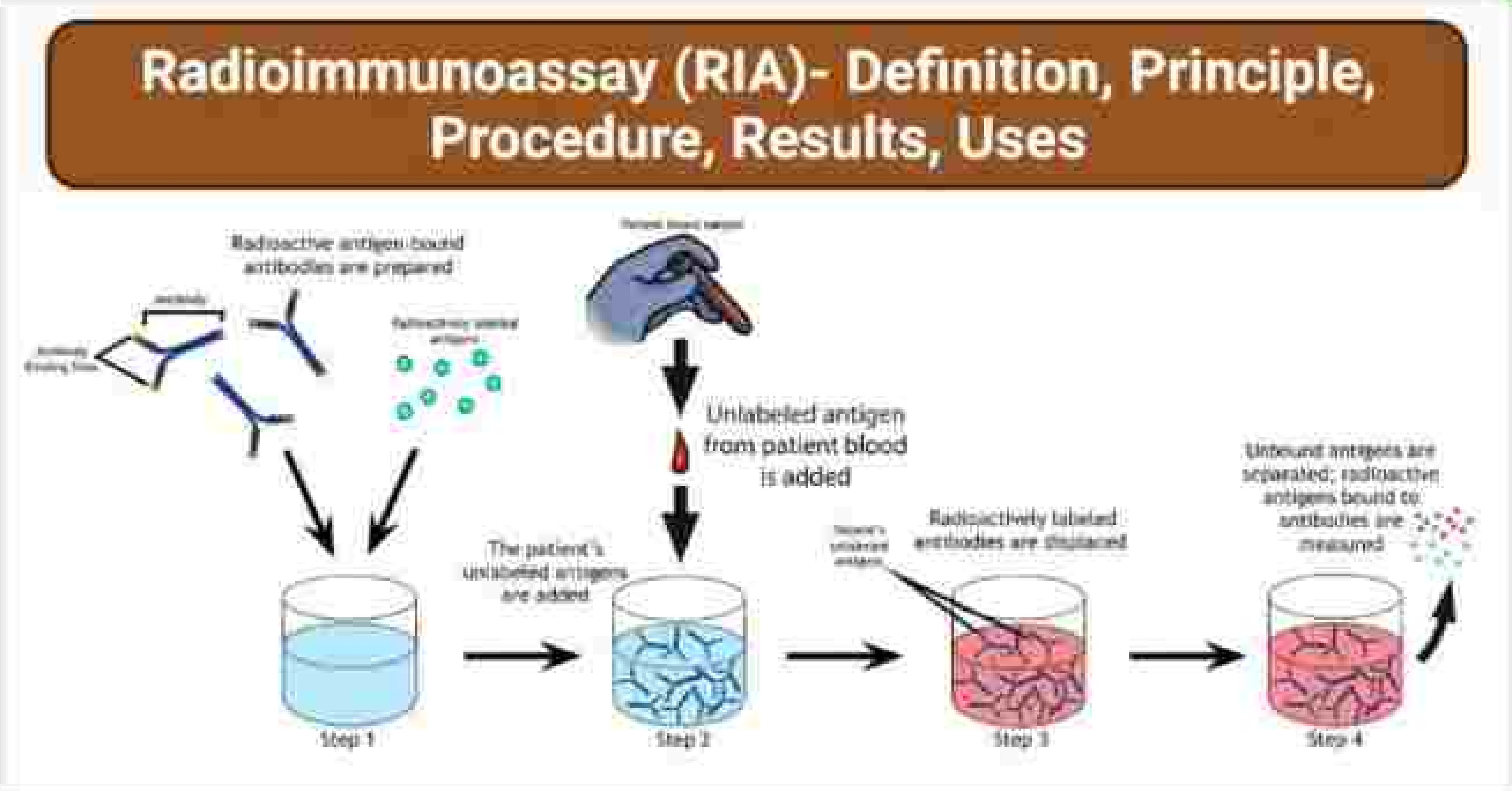
Applications: Biochemistry, distribution studies, metabolite studies, clinical applications, radio immuno assay for detection of Ag/Ab





Radioimmunoassay

- **Radioimmunoassay is one of the sensitive immunoassay techniques which helps in the determination of antigens or antibodies in a sample with the use of radioisotopes**.
- Is an *in vitro* assay that measures the presence of an antigen with very high sensitivity
- RIA was first described in 1960 for the measurement of endogenous plasma insulin by **Solomon Berson and Rosalyn Yalow**.
- *The principle of competitive binding: unlabeled antigen competes with a radiolabeled antigen for binding to an antibody with the appropriate specificity. Radio emission is measured.*
- *The gamma rays from the radiolabeled antigen are measured.*
- Antigen-antibody complexes are precipitated either by crosslinking with a second antibody or by means of the addition of reagents that promote the precipitation of antigen-antibody complexes.
- Counting radioactivity in the precipitates allows the determination of the amount of radiolabeled antigen precipitated with the antibody.
- A standard curve is constructed by plotting the percentage of antibody-bound radiolabeled antigen against known concentrations of a standardized unlabeled antigen, and the concentrations of antigen in patient samples are extrapolated from that curve.
- *Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.*

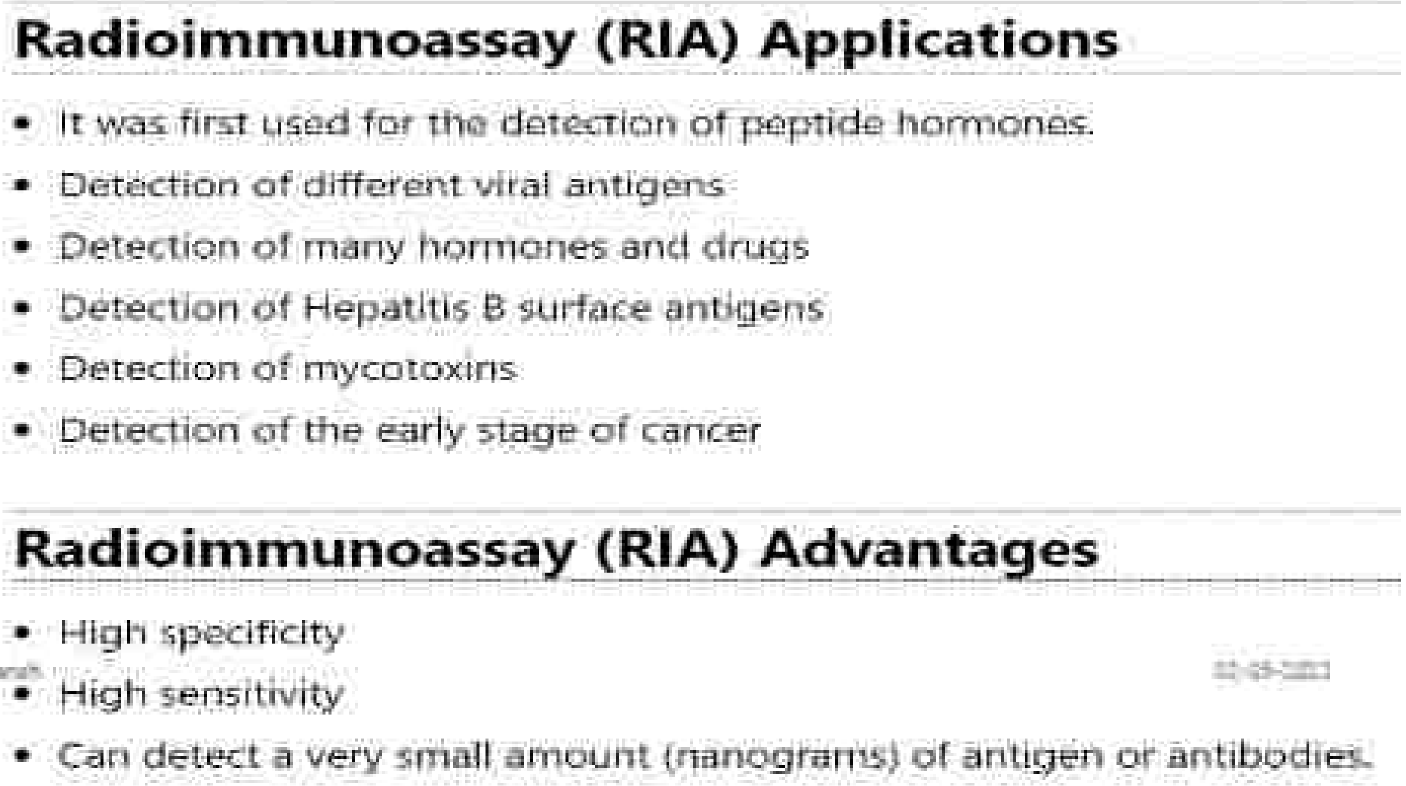


Radioimmunoassay (RIA)- Definition, Principle, Procedure, Results, Uses

Figure: Radioimmunoassay (RIA) Procedure; Image Source: Madeleine Howell-Moroney, 2022.

Radioimmunoassay (RIA) Result Interpretation

- *Initially: t*he labeled antigens will bind to the antibodies hence radioactivity will be maximum.
- If the sample *contains specific antigens of interest*, it will bind to the antibodies releasing labeled antigens *and hence the radioactivity of the solution will decrease*.
- If the radioactivity remains the same, it can be called a negative test.

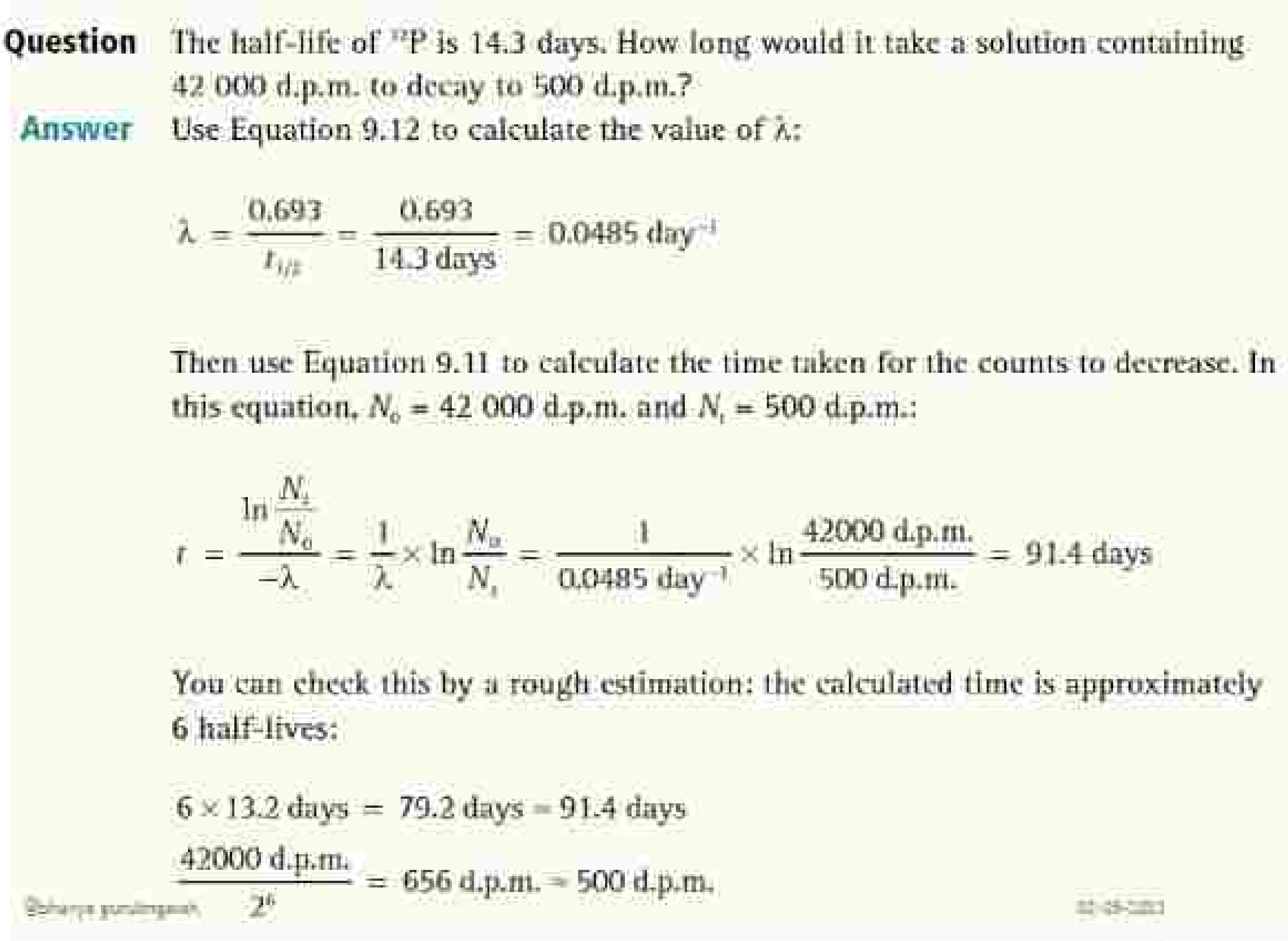


Radioimmunoassay (RIA) Applications

- It was first used for the detection of peptide hormones.
- Detection of different viral antigens
- Detection of many hormones and drugs
- Detection of Hepatitis B surface antigens
- Detection of mycotoxins
- Detection of the early stage of cancer

Radioimmunoassay (RIA) Advantages

- High specificity
- High sensitivity
- Can detect a very small amount (nanograms) of antigen or antibodies.



Question The half-life of ^{32}P is 14.3 days. How long would it take a solution containing 42 000 d.p.m. to decay to 500 d.p.m.?

Answer Use Equation 9.12 to calculate the value of λ:

$$\lambda = \frac{0.693}{t_{1/2}} = \frac{0.693}{14.3\ \text{days}} = 0.0485\ \text{day}^{-1}$$

Then use Equation 9.11 to calculate the time taken for the counts to decrease. In this equation, N_0 = 42 000 d.p.m. and N_t = 500 d.p.m.:

$$t = \frac{\ln \frac{N_t}{N_0}}{-\lambda} = \frac{1}{\lambda} \times \ln \frac{N_0}{N_t} = \frac{1}{0.0485\ \text{day}^{-1}} \times \ln \frac{42000\ \text{d.p.m.}}{500\ \text{d.p.m.}} = 91.4\ \text{days}$$

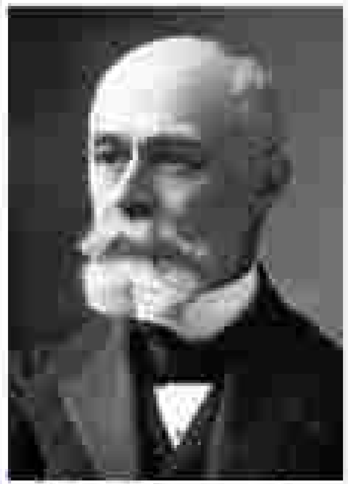
You can check this by a rough estimation: the calculated time is approximately 6 half-lives:

$$6 \times 13.2\ \text{days} = 79.2\ \text{days} \approx 91.4\ \text{days}$$

$$\frac{42000\ \text{d.p.m.}}{2^6} = 656\ \text{d.p.m.} \approx 500\ \text{d.p.m.}$$

Discoveries…….

- the proton number *Z* defines the element, the neutron number *N* defines the isotope and the electron number determines the chemistry.


Henri Becquerel
(Becquerel radiation)
Nobel prize 1903: Marie Curie;
Pierre.


Ernest Rutherford
Protons, α and β
particles
NP: 1908.


Wilhelm Conrad Röntgen
X-rays; NP: 1901


JJ Thomson
electrons; NP: 1906


James Chadwick
neutron; NP 1935

References

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and-radioactivedec
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