

## 4.3 ISOTOPIC TRACERS IN BIO-MEDICAL APPLICATIONS

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### 4.3.1. Historical perspective.

At the beginning of 1910s experiments conducted by F.Soddy gave the first demonstration that most of the elements in nature are composed of atoms identical from the chemical point of view but slightly different in weight. These characteristics were later explained by the fact that each element is identified by the number of protons but may have different number of neutrons, and the term "isotope" was introduced. Just after the discovery of deuterium, for which H.C.Urey was awarded the Nobel Prize in 1934, the idea of using stable isotopes in kinetic/dynamic investigations found useful applications in the early studies on fat metabolism in mice with deuterium by R.Schoenheimer and D.Rittenberg, and continued with studies in nutrition by using  $^{15}\text{N}$ ,  $^{13}\text{C}$  and  $^{18}\text{O}$ . During the successive three decades, with the advent of scintillation counting and the availability of radioactive isotopes, the use of stable isotopes was replaced by the radiotracer technique. The principle of use of radiotracers was actually conceived by G.de Hevesy in the early 1910s, however their widespread use in clinical/biomedical applications really started after the development of the cyclotron as a source for massive (large scale) production, and quickly expanded with the production of radionuclides in nuclear reactors. The stable isotope approach remained confined to light elements as H, O, C and N (in particular for lack of suitable radioisotopes of N and O) in food science with some tentative applications towards other elements, such as the investigations by Lowman and Krivit in the '60s about plasma clearance of iron. Only in the mid 70's stable isotopes regained some interest, due to a greater sensibility of the scientific community towards the use of radioactive substances in healthy volunteer as well as to the availability of new and improved analytical techniques such as ICPMS (Inductively Coupled Plasma Mass Spectrometry) and AMS (Accelerator Mass Spectrometry).

Stable isotopes are now mainly used in the fields of nutrition and physiology for investigations on micronutrients and essential trace minerals, but applications to selected issues can be found. For example, the biokinetics in humans of fallout radionuclides is studied combining the use of stable isotopes as tracers and complementary analytical techniques such as Charged Particle Activation Analysis (CPAA), Thermal Ionization Mass Spectrometry (TIMS) and ICPMS. (more recently SPECT and PET imaging led to widespread use of radiotracers in medical applications).

This note will exclusively deal with applications of isotopic tracers different than imaging purposes, and particularly with metabolic and molecular studies in the life science field.

### **4.3.2. Principles of tracer use.**

Tracer methods find applications in nearly every field of science, be it medicine, biology, physiology, nutrition, toxicology, biotechnology, which are typically life science fields or more technical areas, as physics, chemistry, agriculture, geoscience, engineering, which have now become integral part of every day life.

The common issues for all these applications concern the possibility to trace the entity object of interest, called tracee, that may be a substance, or a component of a substance, like a radical, or an atom. An ideal tracer has the same physical or chemical or biological properties of interest as the tracee, but it presents some peculiar characteristic that enables its detection in the system where the tracee is also present.

The production of an isotopic tracer involves the substitution of one or more naturally occurring atoms in specific positions in the tracee molecule with an isotope of that atom with a less common abundance. Either stable or radioactive isotopes can be used as tracers. Mass differences of isotopes are due to different number of nuclear neutrons, so that the chemical properties are not affected. Both stable and radioactive isotopes of an element take part in the same chemical reactions of the element. The use of a labelled tracer requires the assumption that the labelled molecule, or atom, will not be discriminated from the unlabeled and will trace the position/movement of the unlabelled molecules. Some isotopic effects like evaporation processes or root uptake into plants, can however be observed, especially for light elements and should be taken into account.

### **4.3.3. Aspects in the choice of a stable or a radioactive tracer approach**

The principles of the use of stable and/or radioactive isotopes in tracer studies are similar, however fundamental differences exist which have to be kept in mind in the design of a tracer experiment or in the choice for a tracer application. The optimization process should also take into consideration safety aspects, measurement conditions and equipment requirements in view of the expected or required sensitivity.

The first obvious difference relies in the fact that stable isotopes are non radioactive, that means, in case of in vivo application, no radiation hazard for investigated subjects, with undeniable advantages for particular groups of the population such as pregnant women and newborns. At the same time, this characteristic demands measuring techniques which are usually, even if not strictly always, more complex compared to those used for radioactive ones. For in vitro or non human applications (e.g. molecule labelling, DNA sequencing, environmental studies...) the use of stable and radioactive tracers can be seen as a complementary approach, although the protection of the working personnel exposed to radiation hazard should be taken into account. The choice depends also on the isotopic availability. Monoisotopic elements, like Be, F, Na, Al, P, Mn, Sc, Co, can not be obviously employed, as they are not distinguishable from the intrinsic content. When more stable isotopes are present, as for the five elements of main interest in the life-science field (H, C, O, N, S), the possible tracer will present a modified isotopic composition, with the abundance of one isotope highly enriched with respect to the natural one. Multiple labelling studies as required for example in nutrition and physiology can be performed only when a sufficient number of isotopes and suitable techniques for their simultaneous discrimination are available.

Most of the chemical elements present also one or more radioisotopes, either naturally occurring or artificially produced. Artificial radiotracers may be considered free from any interference due to intrinsic content in the sample, in the other cases a non null blank value can be expected. If this contribution may significantly affect the results, it can be evaluated on the basis of isotopic ratio measurements, which require more accurate and time consuming techniques.

Further factors affecting the choice of radiotracers may be the availability of isotopes with too short ( $^{28}\text{Mg}$ ,  $^{64}\text{Cu}$ ) or too long ( $^{65}\text{Zn}$ ,  $^{45}\text{Ca}$ ,  $^{55}\text{Fe}$ ,  $^{63}\text{Ni}$ ) half-lives in relation to the planned study, or the high costs of the infrastructure and obligatory licences required by the regulations. Stable isotopes, on their side, might be quite expensive (depending on the degree of enrichment desired) and the lower sensitivities of the analytical techniques may demand the administration of significant masses, which might not strictly fulfil the tracer assumption.

Summarizing, for in vitro studies radioactive isotopes may be the tracers of choice, for environmental studies (soils, waters, plants, animals) the advantage of radiotracers should be balanced against possible risks involved for the exposed personnel (including environmental release), whereas in human studies the superior diagnostic information provided must be justified by a soundly recognized benefit for the exposed subject or for the community as a whole. In other cases, stable isotopes should be preferred, and particularly: 1) when the information they provide, combined with a sufficiently refined data analysis, are satisfactory for the study aim; 2) when the exposed subjects (volunteers) are not the ones directly benefiting from the study (research tracer kinetic and metabolism studies in physiology, nutrition, etc.); 3) whereas no suitable radiotracers are available.

#### **4.3.4. Tracer measurement.**

Radioactive tracers can be detected on the basis of their decay properties, as described in 4.1. When the half life is too long or the amounts too small to enable a statistically significant measurement, techniques like the ones used for stable isotopes are needed, which make use of isotopic nuclear properties.

##### **4.1 Through the intrinsic radioactive characteristics.**

The radiotracer is generally the only source of radioactivity present in the sample, apart from the natural background. The decay counting technique, properly corrected for background activity, is therefore sufficient to obtain relative or absolute determinations. The most appropriate radiometric method will obviously be chosen in dependence on the type and energy of radioactive emissions, as well as on its sensitivity grade. If more accurate information are required, such as activity distribution in an inhomogeneous sample or in a living organism, autoradiography or external imaging (e.g., using gamma-cameras as in nuclear medicine) can be employed.

If two or more radioactive tracers are simultaneously present in the sample, simple counting is of no use. The different contributions can be discriminated by repeated counting measurements on the basis of the different half-lives, or by spectrometric measurements on the basis of the characteristic emissions. Routine methodologies involve predominantly the use of gas filled detectors, organic or inorganic scintillators. Indeed, due to the negligible background matrix, the high resolution properties of the solid state detectors are not required for gamma spectrometry.

##### **4.2 Through a stimulated response.**

For the detection of a stable isotope or of a radioisotope with a low activity not suitable for measurement, a characteristic (isotopic) response can be stimulated:

- through application of a series of electrical and/or magnetic fields to the ionized sample, in order to discriminate between atoms or molecules with different  $m/z$  values (thus distinguishing the tracer on the basis of its nuclear mass);
- through induction of nuclear reactions after bombardment of the sample with projectiles of given type and energy (thus distinguishing the tracer on the basis of the decay emissions of the radioactive reaction product).

In the first case, the tracer content in the sample may be determined by measuring the current intensity corresponding to the tracer ions using detectors such as Faraday cups, secondary electron multipliers etc.

As for the induction of nuclear reactions, all sample constituents can be activated (each with its own probability) and therefore the reaction product of interest may be accompanied by a series of other radioisotopes which can cause interference, thus requiring high resolution spectrometric techniques, typically gamma spectrometry with semiconductor diode detectors. It is immediately evident how essential are standard nuclear techniques for a successful application of the tracer methodologies.

For mass spectrometry this is particularly valid when accelerators and detection techniques such as time-of-flight measurements, developed for basic nuclear researches, are employed.

The peculiarity of using such machines lies in the high energy attained by the accelerated ions (in the MeV order of magnitude) which improves significantly the resolution and therefore the accuracy and sensitivity of the technique.

AMS was developed mainly for radiocarbon dating in geochronology studies with a sensitivity many orders of magnitude larger than the usual counting techniques, and more recently applications for the detection of stable or long-lived radioactive isotopes of elements such as H, Be, C, Al, Cl, Ca, Ni, I, U of interest for bio-analytical tracing in the life-sciences have emerged. The accelerators employed for AMS range from simple Van de Graaff or Tandem machines with terminal voltages of few MV to more energetic cyclotrons or linear accelerators.

Another nuclear technique which has been recently applied in bio-tracer studies is neutron scattering analysis for the determination of shapes and dispositions of complex molecules labelled with deuterium. This has been made possible by recent advances in sources and instrumentation for neutron scattering as well as in biotechnology, which have made sample preparation and deuterium labeling easier and cheaper. Also the understanding of the coordinated functions of interacting biomolecules could benefit of a broader application of scattering techniques.

The great potentialities of these techniques with stable isotopes, however, have not been yet fully exploited, mainly due to the complexity and costs linked to the use and maintenance of the large facilities required. Therefore more simple techniques with radiotracers are often preferred, although they imply an exposure to ionizing radiation which is absent or negligible for stable or long-lived radioactive tracers.

#### **4.3.5. Selected applications.**

The two examples shown below give an idea of the different fields which can be investigated using tracer techniques and of the extent of the implications of the results obtained.

##### **4.3.5.1 Molecular and cellular biology**

Radioactive isotopes as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{86}\text{Rb}$ ,  $^{125}\text{I}$  have played and still continue to play a key role in the understanding of the metabolic aspects in cells or bacteria, yeasts, plants and animals (including humans) and in the elucidation of the fundamental properties of genetic material. The radioisotopically labelled metabolites trace the corresponding stable molecules, and autoradiographic or counting measurements provide the information of interest. So, for example,  $^{32}\text{P}$ -dATP is used in the phosphorylation of a protein in order to evaluate its kinasic activity. Similarly  $^{35}\text{S}$  or  $^{125}\text{I}$  label proteins in order to evaluate some specific expressions.  $^{35}\text{S}$ -,  $^{33}\text{P}$ - or  $^{32}\text{P}$ -dideoxynucleotides are used for the synthesis of families of DNA labelled molecules (template DNA) in DNA sequence analysis, and as probes for the detection of specific genes (**Figure 1**). Recently stable isotopes in combination with mass spectrometric techniques have been used for such labelling, especially when the molecules have to be injected into patients for in vivo studies.

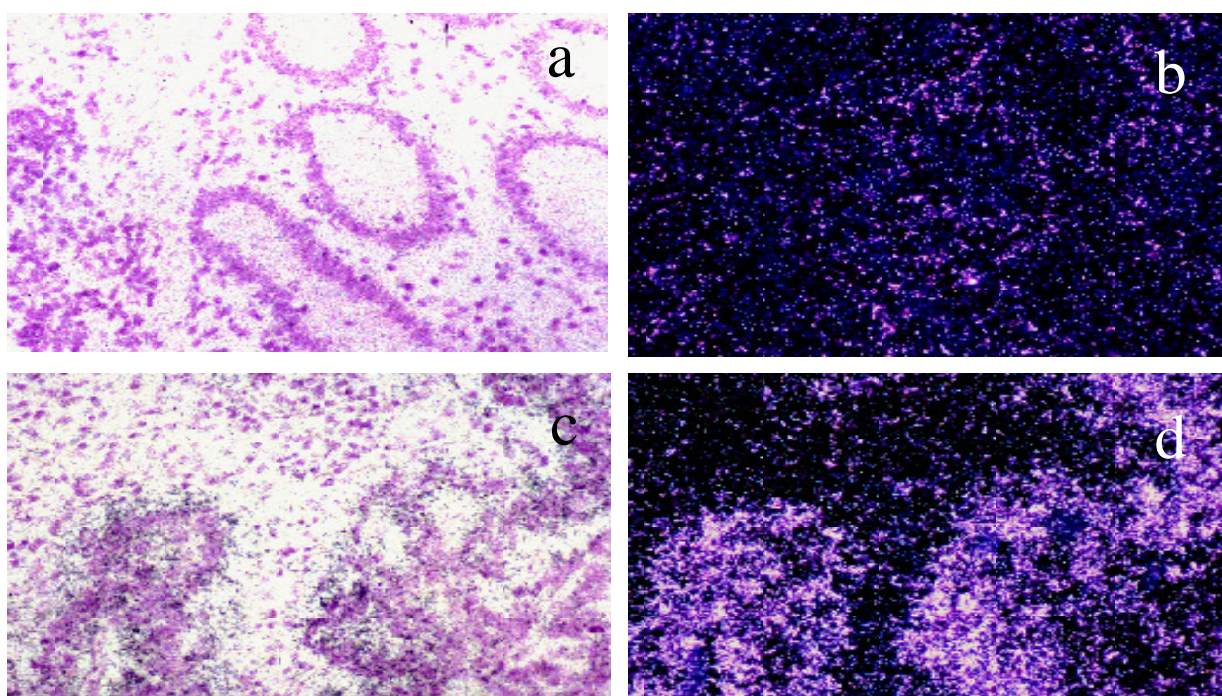


Figure 1: Radioactive in situ hybridization of normal and tumour colon tissue sections. RNA probes containing  $^{35}\text{S}$  UTP were used to detect the presence of the transcript of a specific gene in the two samples. The incorporated radioactivity is detected by autoradiography using silver salt grains to impress a photo film. Panel a and c shows morphology of normal (a) and cancer (c) tissue viewed in bright field under the micro-scope. Switching to dark field (panel b and d) (luminescence of grains is the only source of light) the distribution of the specific RNA in the tissues can now be observed. In the case reported the gene is heavily expressed in the cancer cells as showed by the intensity of the (white) signal.

The incorporation of  $^{86}\text{Rb}$  into glial and neuronal cells is a method to trace the potassium entering the cell via the Na/K pump and therefore to monitor the Na<sup>+</sup>/K<sup>+</sup> ATPase activity in these cells, which is an important information in the study of neurodegenerative diseases. The value of the uses of tracers in genetics will continue to improve, particularly considering that gene therapy is now beginning to find its way into ordinary clinical application.

#### 4.3.5.2 Elemental kinetics

The biokinetics of essential elements, trace elements, micronutrients or any other elements of interest in nutrition, physiology, toxicology can be studied by tracer method. As already pointed

out before, the use of stable tracers is the ethically justifiable choice when dealing with healthy volunteers, although this could mean less information and/or more cumbersome measurements than with radiotracers. Therefore a sound experimental design is required. The double (or multiple) tracer technique, for example, consists in the simultaneous administration of two (or more) tracers through different pathways (typically, one oral and one intravenous tracer), and thus permits to obtain dynamical pictures of relevant processes such as the intestinal absorption or the main excretion pathways (*Figure 2*).

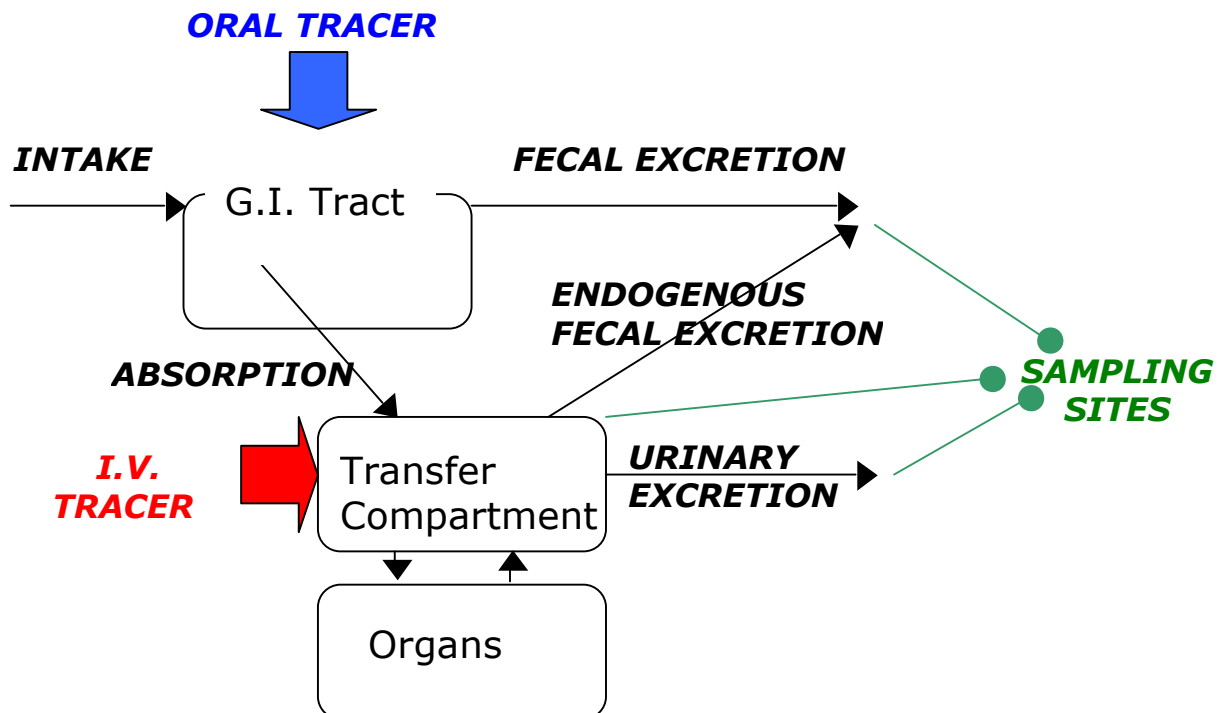


Fig. 2 - Simplified scheme of the distribution of an element in the organism, as can be studied using the double stable tracer technique. One tracer is administered orally, the other tracer is injected intravenously. Blood, urine and feces samples are then collected and analyzed, in order to characterize the processes of interest.

Many analytical approaches are used for the determination of the stable tracers in biological samples, and many are the works which can be found in literature about the study of biokinetics of nutrient and non-nutrient elements. For some elements such as iron, molybdenum, ruthenium, zirconium, the combination of stable isotopes, charged particle activation analysis and thermal ionization mass spectrometry has enabled to collect, for the first time ever, a detailed picture of the kinetics in blood plasma and of the renal elimination process. It has been therefore possible to revise the existing models, in order to provide a more realistic description of the biokinetics of ingested material. Radiation protection is, for example, a field which may greatly benefit from this improved realism, since the revised models may enable a more correct interpretation of control measurements in persons suspected of contamination and provide a sound support for the implementation of effective protective actions in case of radiological emergencies.

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